RESPONSES TO STIMULATION OF ATRIAL RECEPTORS

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Doctor of Science

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1987

ABSTRACT

Complex sensory nerve endings, functioning as stretch receptors exist within the vessel walls of both the high and low pressure vascular systems. The reflex effects of stimulation of receptors in the high pressure system (arterial baroreceptors) are known in some detail; less is known regarding the function of receptors in the low pressure system. The work reported here is concerned with the reflex responses to stimulation of unencapsulated nerve endings found within the endocardium of the left atrium and having myelinated afferent fibres in the vagus nerves (left atrial receptors).

Evidence is presented that stimulation of left atrial receptors leads to complex and highly specific reflex responses. Stimulation of left atrial receptors causes a reflex increase in heart rate which is mediated mainly through the cardiac sympathetic nerves. There is also a reflex decrease in renal vascular resistance, although the resistance in other vascular beds remains unaltered. Stimulation of atrial receptors did not cause any change in respiratory rate or tidal volume. Stimulation of atrial receptors is associated with a diuresis and under some circumstances a natriuresis. At least a part of the diuresis appeared to be dependant on a decrease in the concentration of vasopressin in the plasma. More recently it became possible to measure the plasma concentration of vasopressin by radioimmunoassay and it was shown that stimulation of atrial receptors caused a reflex decrease in plasma vasopressin concentration. The recent demonstration that the atrial myocytes produce a natriuretic peptide (atrial natriuretic factor), which is released during atrial distension, adds another dimension to the control of blood volume. The release of this peptide was not influenced by atrial receptor stimulation.

The atrial receptors appear to provide one mechanism by which either total blood volume, or the filling of the heart may be detected. The reflex increase in heart rate induced by atrial receptor stimulation may act along with the "Starling Mechanism" to maintain heart volume constant. Continued stimulation of atrial receptors, acting through the renal nerves and a reduction in plasma vasopressin, will promote the excretion of water and salt and tend to reduce blood volume.

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J. Physiol. (1961), **159**, *pp.* 87–100
With 8 *text-figures*
Printed in Great Britain

THE MECHANISMS BY WHICH DISTENSION OF THE LEFT ATRIUM PRODUCES DIURESIS IN ANAESTHETIZED DOGS

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(*Received* 1 *May* 1961)

Recent reviewers primarily interested in renal (Smith, 1957) and cardiovascular physiology (Neil, 1960) have accepted a theory that distension of the left atrium of the heart sets up afferent impulses in the vagus nerves which decrease the release of antidiuretic hormone from the neurohypophysis, and so cause diuresis. The theory is within the general conception that the renal excretion of water and electrolytes is partly governed by the volume in some fluid compartment of the body. Support for this role of atrial receptors has been derived from the experiments of Henry, Gauer & Reeves (1956), who found that in anaesthetized dogs inflation of a balloon in the left atrium caused an increase in urine flow with a time course and other characteristics which could be accounted for by a diminished release of antidiuretic hormone. The paper of Henry *et al.* (1956) contains little evidence to support this interpretation. In view of the theoretical importance attached to them the experiments have been repeated and extended in this paper; whilst the main results are confirmed, the small and variable size of the response is emphasized and additional observations make untenable the explanation that the diuresis is due to decreased release of antidiuretic hormone. A preliminary account has already been published (Ledsome, Linden & O'Connor, 1961).

METHODS

The experiments were carried out as described by Reeves, Henry & Gauer (1956). Dogs of 10–15 kg were given 15 mg morphine sulphate by subcutaneous injection and 1 hr later were anaesthetized by the intravenous infusion of 1% chloralose (British Drug Houses; 10 ml. = 0·1 g/kg) in sodium chloride solution 0·6 g/100 ml. Subsequently during the experimental procedures a steady state of light anaesthesia was maintained by the infusion every 10 min of either 1 or 0·5% chloralose, about 1 ml./kg. Each ureter was catheterized through a flank incision into the peritoneal cavity, and urine volume was measured every 10 min (Henry *et al.* (1956) collected urine from a urethral catheter). With the animal under positive-pressure ventilation from a Starling 'Ideal' Pump, the chest was opened in the left fifth intercostal space, and a balloon inserted into the left atrium through the appendage in which it was secured by a ligature. The chest was closed, air expelled through a drainage tube in the 7th intercostal space and normal respiration restored. The operative

procedures were completed in about 2½ hr and 2 hr were then allowed to elapse before the first experimental tests. Rectal temperature was maintained between 37·5 and 38·5° C.

In experiments in which one kidney was denervated the right kidney was approached through an incision 1 cm below and parallel to the 12th rib. All fascial connexions were divided between ligatures, until the only attachments of the kidney were the blood vessels and the ureter. Obvious nerve trunks were severed, the vessels were stripped free of fascia and the ureter catheterized and divided below the catheter.

The atrial balloon was made from a 2 cm length of the finger of a surgical glove tied over the end of a polyethylene tube of 2 mm bore. A second polyethylene tube of 1 mm bore was tied alongside the atrial balloon for the measurement of mean left atrial pressure. The balloon was judged to be satisfactorily placed when it lay with its base included in the ligature around the tip of the atrial appendage. It was expanded at each test by 1 ml. saline/kg body weight, or less if the atrial pressure had already risen by about 20 cm H_2O.

Femoral arterial blood pressure and mean left atrial pressure were recorded by capacitance manometers operating ink writers; the frequency response of the channel recording femoral blood pressure was flat (±5%) to 20 c/s. In some experiments respiration was recorded from a manometer connected to a cannula in the right pleural cavity or in the trachea.

Vasopressin was infused through a cannula threaded through a femoral vein so that its tip lay in the inferior vena cava. A motor-driven syringe delivered 0·14 ml. of 0·9% NaCl solution/min to which was added vasopressin (Pitressin; Parke, Davis and Co. batch LZ 1094C or LY 258J) to give the stated rate of infusion. The batches of Pitressin used contained a mixture of lysine vasopressin and arginine vasopressin with the lysine vasopressin probably predominating (personal communication, Parke, Davis and Co.), and were labelled as containing 20 u./ml. of pressor activity. In this paper, 1 m-u. means 0·00005 ml. of Pitressin.

Urine was analysed for sodium and potassium by flame photometry, and for ammonium by the method of Conway (1957). Freezing-point depression was determined by means of a standard Beckmann thermometer graduated to 0·01° C and the osmolar content of the urine was calculated. pH was determined approximately by indicator papers.

RESULTS

Time course and size of the diuresis

Figure 1 shows the result of an experiment similar to those illustrated by Henry *et al.* (1956), in which a balloon was inflated in the left atrium and the inflation maintained for 30 min. In response to the obstruction produced by the balloon the mean pressure in the femoral artery fell 20 mm Hg, there was a rise of 20 cm H_2O in mean left atrial pressure, heart rate rose by about 20 beats/min, and respiration increased by 10 breaths/min. Urine volume from the two kidneys increased gradually from 0·3 ml./min to reach 1·9 ml./min after 30 min.

To present the results of all experiments in which the balloon in the left atrium was inflated for 30 min the results are plotted in Fig. 2 in the form used by Henry *et al.* (1956); the mean rate of urine flow (ml./min) for the 40 min preceding the test and the 40 min following the diuresis (i.e. the mean of urines 1, 2, 3, 4, 9, 10, 11, 12 in Fig. 1) was regarded as the control rate, to be compared with the rate during the diuresis (i.e. mean of urines 6, 7, 8 in Fig. 1). Forty-two balloon inflations were made in

eighteen dogs. Figure 2 shows the results of twenty-four inflations in which the vagus nerves were intact and no infusion of vasopression was made. The continuous line separates two observations in which urine volume (ml./min) decreased, from twenty-two in which there was an increase. The interrupted lines separate nine observations when the urine volume during the diuresis was 1–2 times that during the control period,

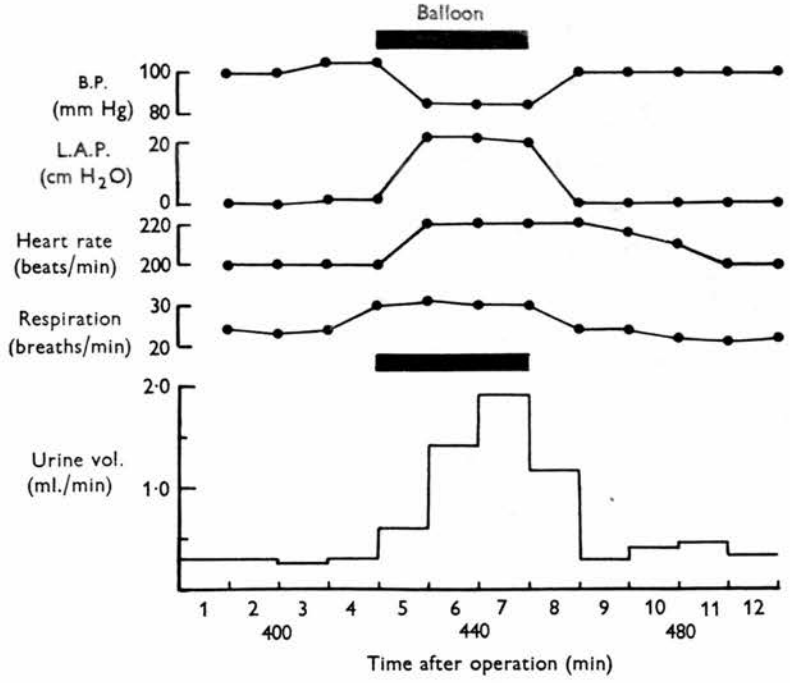

Fig. 1. Effect of inflating a balloon in the left atrium, for the 30 min indicated by the solid bar. From above downwards, femoral arterial pressure, left atrial pressure, heart rate, respiratory rate and urine volume.

six observations 2–3 times, and seven observations more than 3 times the control volume. The scatter is similar to that shown in the corresponding figure of Henry *et al.* (1956). The small size of the diuresis is illustrated by the fact that on only two occasions did the urine volume exceed 2 ml./min compared with 6 ml./min usually attained during water diuresis in the conscious dog.

During control periods mean left atrial pressure was between 0 and 17 cm H_2O and rose during the inflations by 12–32 cm H_2O. Mean arterial blood pressure in the control periods was 75–145 mm Hg and fell during the obstruction of the left atrium by 0–30 mm Hg. The average mean fall in arterial pressure in the twenty-four experiments was 10 mm Hg (S.E.M. $\pm$ 1·3). The heart rate was between 75 and 200 beats/min and increased

during the inflations by 10–120 beats/min, the largest increases being on the occasions when the control rates were low. The respiratory rate was 10–45 breaths/min and was increased by about 15 breaths/min.

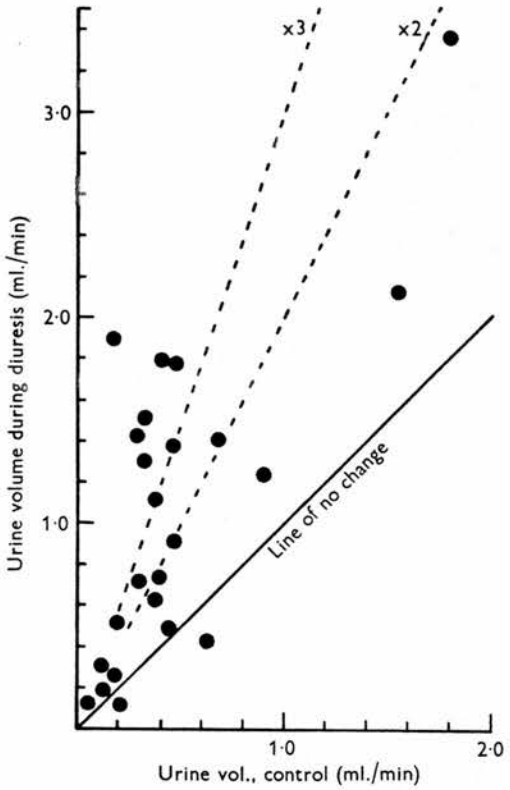

Fig. 2. Results of twenty-four balloon inflations in eighteen dogs. Urine volume during the diuresis compared with urine volume during the control periods. The interrupted lines indicate diuresis of two and three times the control values.

The results in Fig. 2 leave no doubt that inflation of the balloon did produce a diuresis, but detailed examination of the response must be confined to examples where the diuresis was at least twice the resting flow. A smaller response (e.g. increase of urine flow from 0·4 to 0·7 ml./min) could not clearly be recognized against the variations within control periods, and description of its time course or of the changes in urinary composition was impossible.

If inflation of the balloon in the left atrium was maintained for periods longer than 30 min the diuresis began to decrease despite the continued distension of the atrium. Figure 3 shows an experiment in which a balloon was inflated for 70 min. Urine volume increased to reach a maximum

after 50 min and thereafter decreased, although the high left atrial pressure, low arterial blood pressure and raised heart and respiratory rates were maintained. This was the most prolonged diuresis observed and on other occasions the diuresis began to fall off after 30–40 min; Henry *et al.* (1956) show a similar record. Thus Fig. 1 could be misleading in that it might give the impression that obstruction of the left atrium caused the urine flow to rise to a plateau which would be maintained until the balloon was deflated.

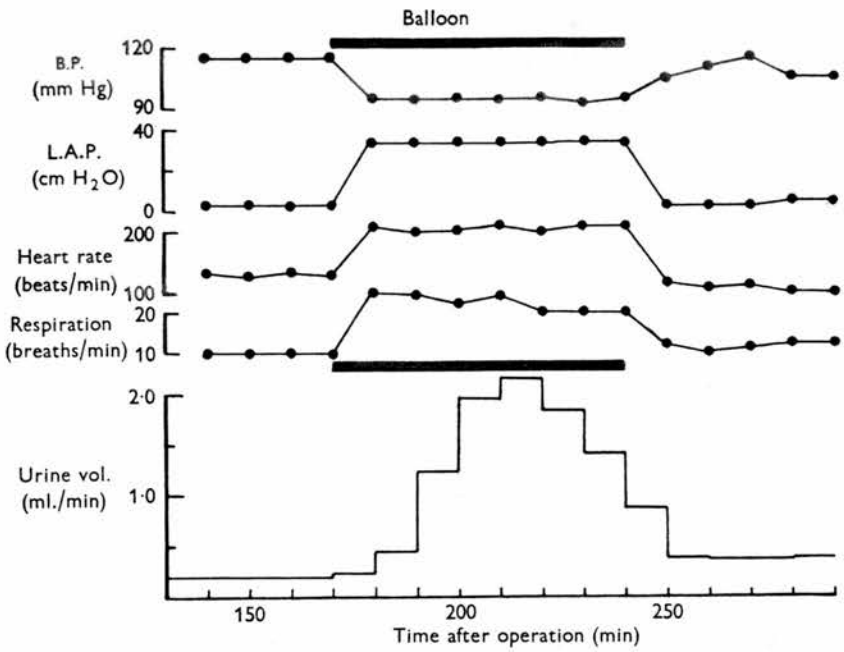

Fig. 3. Effect of inflating a balloon in the left atrium for 70 min; conventions as in Fig. 1.

To obtain a diuretic response to inflation of the balloon, Henry *et al.* (1956) thought it necessary to have the chest closed and the animal breathing spontaneously, and this technique was followed in the experiments so far described. However, in each of four experiments in which the chest was left open and respiration maintained by positive-pressure ventilation, a diuretic response was obtained. Figure 7 shows such an experiment in which there were typical changes in arterial pressure, left atrial pressure, and heart rate, and a diuresis of four times the control level, artificial respiration being maintained constant at 18/min throughout.

Henry *et al.* (1956) stated that at least 2 hr should elapse after the operation before inflating the balloon, and usually we followed their practice.

However, diuresis has been observed on each of two occasions when a balloon was inflated within an hour of the operation; Fig. 7 illustrates this finding, the diuresis being produced 40–70 min after completion of the operation.

Infusions of dog blood, plasma or dextran (6 g/100 ml. in NaCl solution 0·9 g/100 ml.; 'Dextraven', Benger Laboratories Ltd.) were occasionally given during the experiments as supportive measures without enhancement of the diuretic response to balloon inflation, although Henry *et al.* (1956) claimed that infusions of bovine albumin or dog blood did improve the response. However, transient increases in urine volume occurred without inflation of the balloon after seven out of eleven infusions, as in the example in Fig. 6. On three occasions a diuresis occurred spontaneously without obvious association with any experimental procedure. The time course and magnitude of these diureses was similar to the effects of inflation of the balloon in the left atrium.

Excretion of solutes

Figure 4 shows the results of analysis of the urine from the right kidney. In response to inflation of a balloon in the left atrium, urine volume increased from 0·12 to 0·8 ml./min, an increase of about 6 times, whilst during the same period the excretion of the solid constituents in the urine increased by less than 70%; the concentration of the various solutes fell to about one fifth of the control values. Analysis for sodium and potassium was made in nine tests where the diuresis was 2–6 times the control flow and showed similar small changes in the excretion of these substances. Total urine solutes were estimated by means of the freezing-point depression in five tests in which the urine volume during the diuresis rose to 3·5 times the control volume, whilst the excretion of the solutes only increased by 20%. The pH in the control periods was about 7·0, and was not altered during the diuresis; increases in ammonium excretion were small in two experiments where this was examined.

Effect of infusion of vasopressin

Figure 5 shows the effect of inflation of a balloon in the left atrium by exactly the usual procedure, but during an infusion of vasopressin. Infusion at a rate of 0·025 m-u./kg/min started 50 min before the test and continued until 30 min afterwards had no effect on urine volume and in no way modified the diuretic response, which was similar to the diureses in Fig. 5 when no vasopressin was infused. A diuretic response was observed on each of five occasions when a balloon was inflated during the infusion of 0·025 m-u./kg/min. Larger infusions of vasopressin of up to 0·1 m-u./kg/min were also used. Figure 6 depicts the effects produced by two

balloon inflations made during infusion of vasopressin at a rate of 0·1 m-u./kg/min. On each occasion a diuresis of about twice the control volume was obtained. Infusion at this rate was without obvious effect on control urine volume, left atrial pressure or arterial blood pressure.

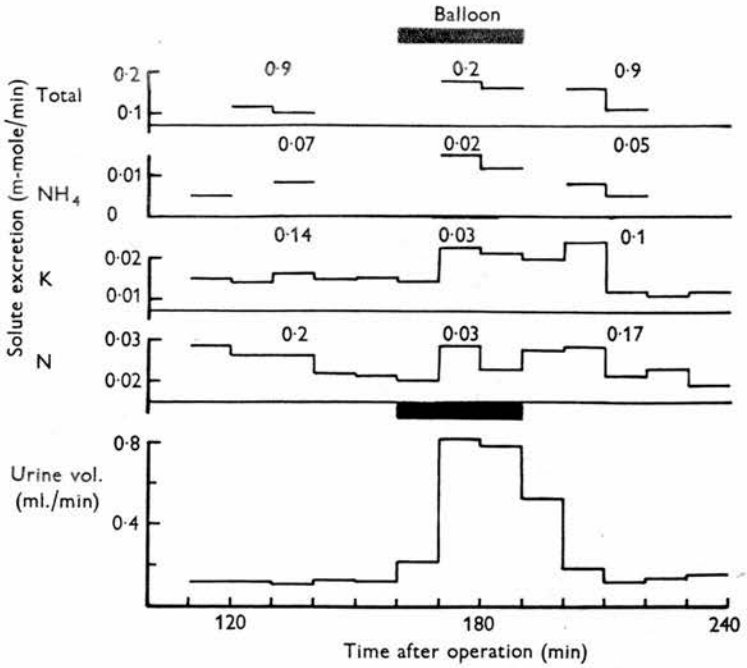

Fig. 4. Composition of urine and rates of excretion from right kidney during inflation of a balloon in the left atrium. From above downwards, urine solute excretion—total solute, ammonium, potassium and sodium—and urine volume (ml./min). Figures written above each line show urine concentration (M).

Since it was uncertain whether the batches of Pitressin contained lysine or arginine vasopressin, they were tested on a conscious dog. Infusion at 0·009 m-u./kg/min was begun and 400 ml. of water given by stomach tube 5–10 min later. In the absence of vasopressin water diuresis always resulted but 0·009 m-u./kg/min of either batch of Pitressin was adequate to prevent the diuresis until after the infusion was ended. Thus the rate of infusion of vasopressin in Fig. 5 was at least 2·5 times and in Fig. 6 was 10 times the rate needed to abolish water diuresis in the conscious dog.

Effect of vagotomy

In eight tests on five dogs a balloon was inflated in the left atrium after section of the vagus nerves in the neck and no diuresis resulted in any of these tests (Fig. 7). In five of the tests there was a small fall in urine flow.

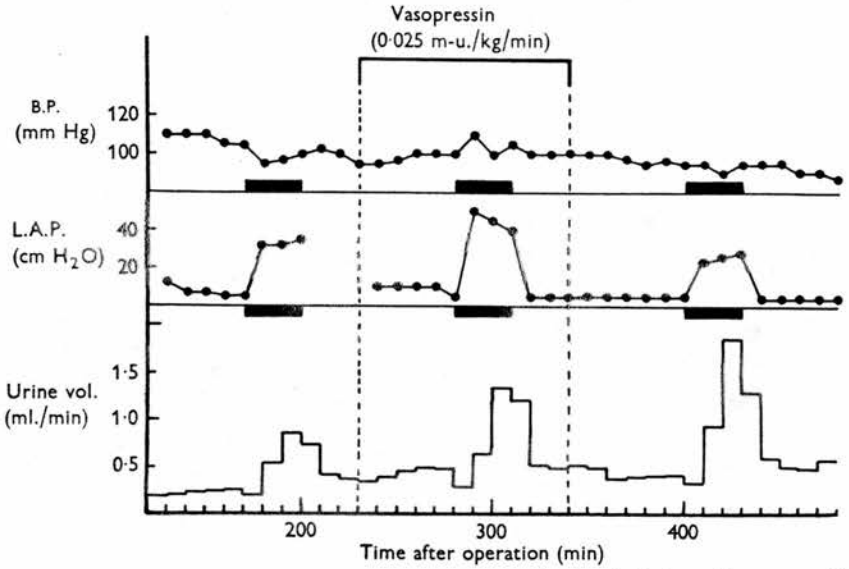

Fig. 5. Diuresis produced by balloon inflation during infusion of vasopressin. Vasopressin infusion 0·025 m-u./kg/min was started 50 min before and continued until 30 min after the second inflation. From above downwards, femoral arterial pressure, left atrial pressure and urine volume.

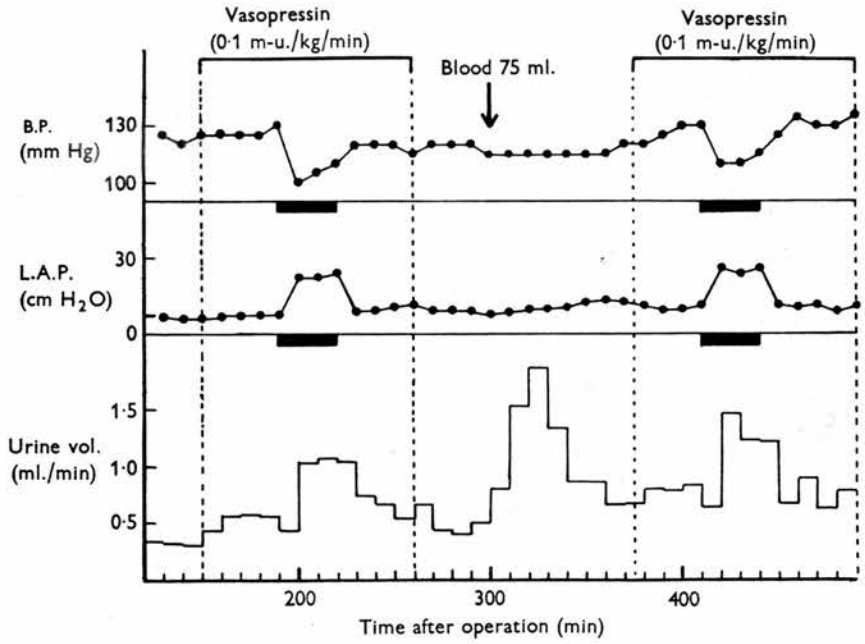

Fig. 6. Two balloon inflations during infusion of vasopressin 0·1 m-u./kg/min. Conventions as in Fig. 5. A diuresis following an infusion of 75 ml. of dog blood is also shown.

The balloon was inflated at times varying from 40 min to 4 hr after vagotomy; in two experiments the vagi were cut immediately after the operation, whereas in three (e.g. Fig. 7) a typical diuresis was elicited in a test inflation before the vagi were cut. When the dog was breathing naturally vagotomy caused changes in the rate and depth of respiration; in Fig. 7 the experiment was under positive-pressure artificial ventilation which was maintained unchanged throughout. Heart rate was high after vagotomy and did not change when the balloon was inflated, but changes in left atrial pressure and femoral arterial pressure were similar to those seen during the test before vagotomy.

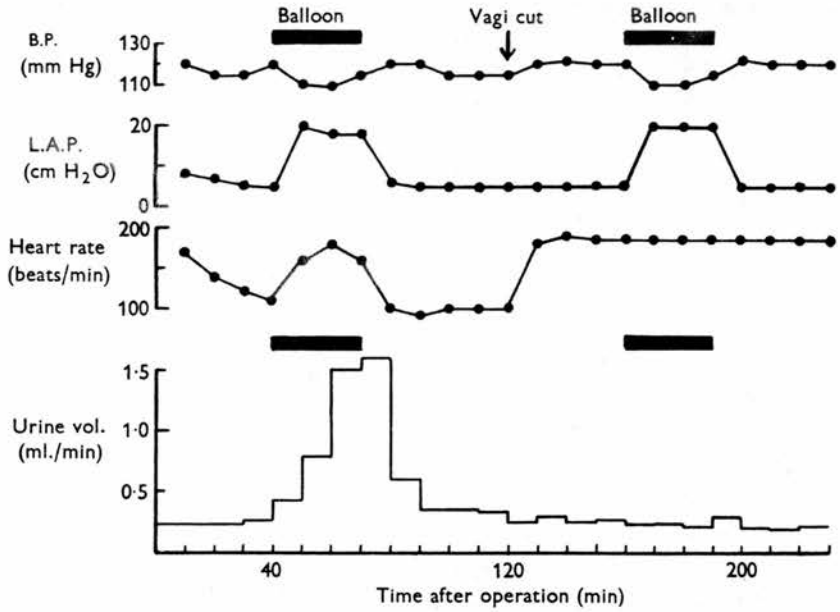

Fig. 7. The effect of balloon inflation before and after section of the vagus nerves. From above downwards, femoral arterial pressure, left atrial pressure, heart rate and urine volume. In this animal the chest was open and positive-pressure artificial ventilation was maintained unchanged throughout. Vagi cut at time marked by the arrow.

Effect of denervation of a kidney

The right kidney was denervated in four dogs and 3–5 hr later, when urine flow from both kidneys was steady, a balloon was inflated in the left atrium. Figure 8 shows the result of one experiment. During the control period urine volume from the denervated kidney was about 50 % greater than from the intact kidney, and when the balloon was inflated the urine volume doubled in both kidneys. The typical diuresis was also produced in a denervated kidney in each of the three other experiments.

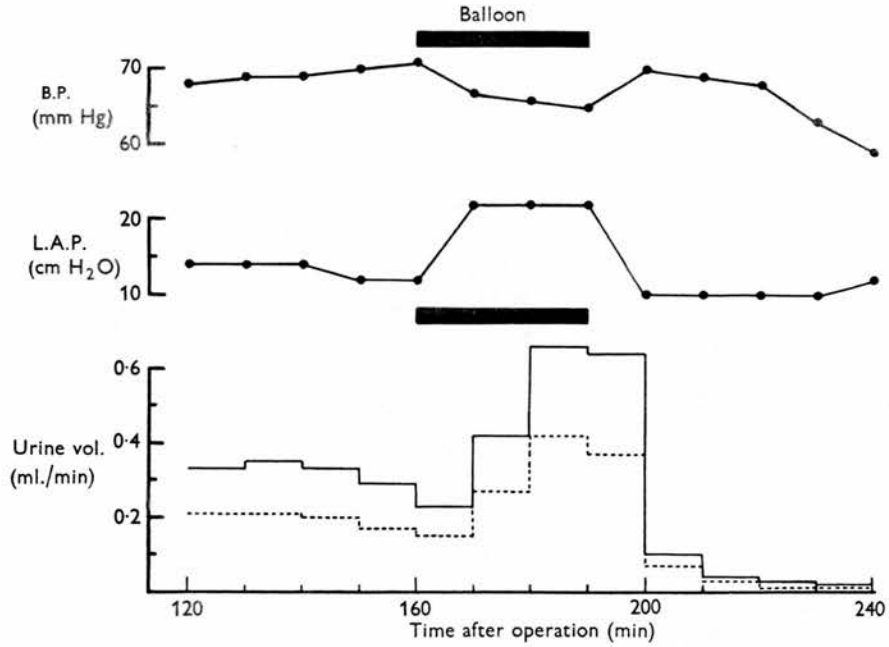

Fig. 8. Effect of balloon inflation on a denervated kidney. From above downwards, femoral arterial pressure, left atrial pressure and urine volume. —— urine volume from denervated (R) kidney; ----- urine volume from innervated (L) kidney. Chest open and positive-pressure artificial ventilation maintained unchanged throughout.

DISCUSSION

The main experimental finding of Henry *et al.* (1956) has been confirmed; obstruction of the left atrium in dogs under chloralose anaesthesia produced an increased flow of urine. The diuresis was transient, and small when compared to water diuresis in conscious dogs, and it was never possible to predict from the heart and respiration rates, arterial and left atrial pressures recorded during the control periods whether the effect of inflating the balloon would be comparatively large or virtually absent. Technical procedures mentioned by Henry *et al.* (1956) as likely to ensure a good response proved unreliable and no way was found of obtaining consistent responses. However, despite the occasional spontaneous diuresis the results in Fig. 2 were sufficient to establish that inflation of the balloon *did* produce a diuresis, but its size and variability made detailed investigations difficult and attempts to explain the mechanisms involved must be largely speculative.

Henry *et al.* (1956) and Henry & Pearce (1956) suggest that the first stage in the mechanism is the stimulation of receptors in the left atrium.

The existence of receptors stimulated by distension of the intrathoracic parts of the circulation is now well established, and in the dog they have been found in the pulmonary arteries (Coleridge & Kidd, 1960) and at the junctions of the venae cavae and pulmonary veins with the atria (Coleridge, Hemingway, Holmes & Linden, 1957). Receptors in the pulmonary arteries and left atrium presumably discharged more frequently when a balloon was inflated to obstruct the mitral opening; Henry & Pearce (1956) recorded the discharge from a receptor in the left atrium. Also Henry & Pearce (1956) observed no diuresis when the left atrium was obstructed with the vagi blocked by cold, and we were unable to produce a diuretic response to balloon inflation after vagal section (Fig. 7). However, section of the vagi involves the interruption of many different efferent and afferent nerve fibres and cannot be regarded as merely interrupting the afferent limb of a specific reflex. Section of the vagi produces a very great disturbance of circulation and respiration and absence of the diuresis might be due to such general effects rather than the loss of the afferent impulses from intrathoracic receptors. Since we could not define circumstances under which the response appeared, it is impossible to say whether the general state of the animal after vagotomy was or was not such that diuresis would be expected. Thus it is possible that increased discharge from intrathoracic receptors is a necessary part of the sequence by which obstruction of the left atrium produces diuresis, but no real evidence either for or against such a hypothesis has yet been presented.

Whether the mechanism is a reflex with its afferent fibres in the vagi or the diuresis is produced by some other mechanism, some agent must eventually reach and act on the kidney to produce the increase in the urine flow. Two possibilities appear to be excluded by our experiments. First, the diuresis occurred in the denervated kidney (Fig. 8) and therefore was not due to changes in the activity of the renal nerves. Secondly, the diuresis occurred during infusions of vasopressin (Figs. 5, 6), and therefore was not due to decreased release of antidiuretic hormone of the neurohypophysis. The characteristics of the urine in Fig. 4 do not match the effects of any of the agents known to have important effects on the volume and composition of the urine (O'Connor, 1961). If the diuresis were due to dilution of plasma protein or increased renal blood flow, a greater increase in sodium excretion would be expected. If it were an osmotic diuresis the excretion of osmotically active solutes should increase nearly in proportion to the increase in urine volume. If a large amount of an organic anion entered the plasma and was excreted in the urine then the urine would become acid and contain ammonium. The time course and nature of the diuresis is unlike any effect to be expected from an altered rate of release of adrenal cortical steroids. The usual effect of increased

release of adrenaline or noradrenaline is decreased urine flow and decreased excretion of sodium (O'Connor, 1961). Thus the agent acting on the kidney to produce the effect of Fig. 4 cannot be recognized by the characteristics of the urine. However, it should be realized that the diuresis in the experiments of this paper was small, transient and in animals under chloralose anaesthesia subjected to a severe operation. The urinary effects of the agents known to act on the kidney (O'Connor, 1961) have been described from experiments in which large effects persist in conscious animals; comparison between the two types of experiments may be misleading.

When the experiments of Henry *et al.* (1956) are cited in the literature on volume receptors, it is usually assumed that the diuresis is due to a decreased release of antidiuretic hormone from the neurohypophysis. Henry *et al.* (1956) offered no evidence for this suggestion, which is based on a comparison with a somewhat similar diuresis produced by breathing from a reservoir containing air at pressure less than atmospheric (Gauer, Henry, Sieker & Wendt, 1954; Sieker, Gauer & Henry, 1954). Our finding that the diuresis occurs during infusion of vasopressin is in obvious conflict with the accepted explanation, and a detailed discussion of the evidence concerning the activity of the neurohypophysis in dogs under chloralose anaesthesia therefore seems necessary.

Theobald (1934) gave to dogs by stomach tube anaesthetic doses of chloralose dissolved in 300 ml. of water. The animals 'fell asleep' during the course of the water diuresis and the diuresis could be inhibited by intravenous injection of the same doses of vasopressin as inhibit water diuresis in the conscious animal. Apparently immediately after induction of chloralose anaesthesia there is no abnormal release of antidiuretic hormone from the neurohypophysis, and the kidney tubules respond normally to vasopressin. The conditions in our experiments were very different in that chloralose anaesthesia had been maintained for at least 3 hr and a major surgical operation carried out. Water, injected intravenously or given by stomach tube to these animals, did not produce water diuresis and Verney (1929) found that under similar conditions water was absorbed from the stomach but no diuresis resulted. In experiments like those of this paper Baïsset, Douste-Blazy, Montastruc & Valdiguié (1957) describe antidiuretic activity in extracts of jugular-vein blood, assayed as equal to 0·4 m-u. vasopressin/ml. serum, which would indicate the release of about 40 m-u./min, as compared to the release in normal conscious dogs of about 0·1 m-u./min (Verney, 1947): premedication with morphine might cause some release of antidiuretic hormone (Duke, Pickford & Watt, 1951). Eisen & Lewis (1954) indicate that antidiuretic hormone may be released during operations in human subjects and it was for this reason that Henry *et al.* (1956) waited 2–4 hr after completion of

the operation before testing the effect of inflating the balloon in the left atrium; we have obtained the diuresis only 40 min after the operation (Fig. 7). It is not clear whether water diuresis fails to occur under these conditions because of a continual release of antidiuretic hormone or some other substance or because of some defect in the kidney itself. Chloralose in conjugated form is excreted in the urine (Kochman, 1923) and may well affect the tubular cells; in our experiments the urine contained a reducing substance which, with Benedict's quantitative reagent, did not give the colour change characteristic of glucose. Since water diuresis cannot be established under the conditions of our experiments it is impossible to test whether the kidneys respond normally to injections of vasopressin and there is no standard by which to assess the effective dose. The diuresis caused by obstruction of the left atrium occurred during infusion of vasopressin at 10 times the rate needed to stop water diuresis in conscious animals and is therefore not produced by any *normal* function of the neurohypophysis. If the neurophypophysis is part of the mechanism producing diuresis in the experiments in Figs. 5 and 6, the sensitivity of the kidney to antidiuretic hormone must also be abnormal, and the effect is so far removed from normal function of the neurohypophysis as to have no relevance in the study of the normal control of urinary volume.

The experiments of Henry *et al.* (1956) are not usually cited as evidence for the general theory of the control of urinary volume by volume receptors. Henry *et al.* (1956) carried out their experiments because study of the effects in dogs and man of breathing from a reservoir at pressure less than atmospheric had convinced them that intrathoracic volume receptors did produce inhibition of the neurohypophysis and so diuresis, and their object was to determine the site of the receptors. Discussion in this paper of the experiments of Henry *et al.* (1956) certainly indicates that they can have no general application until the causative mechanisms can be elucidated, and they should not be used in interpreting the effects of negative-pressure ventilation or other procedures which affect urine flow. Likewise this paper provides no direct evidence which denies either the general conception of volume receptors or the interpretation of the effects of negative-pressure ventilation as due to inhibition of the neurohypophysis; these ideas can be criticized on other grounds (O'Connor, 1961).

SUMMARY

1. In confirmation of the findings of Henry *et al.* (1956), distension of the left atrium of dogs under light chloralose anaesthesia produced a diuresis which was small, transient and variable in size.

2. During the diuresis there was at most a small increase in the excretion of sodium, potassium or total urinary solutes.

3. Diuresis was produced on balloon inflation after renal denervation.
4. The diuresis was also produced during infusion of vasopressin at a rate adequate to inhibit water diuresis in the conscious dog.
5. The diuresis did not occur after section of the vagus nerves.
6. The mechanisms involved cannot be satisfactorily explained.

We wish to thank Mr J. Brook for his excellent technical assistance.

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With 7 text-figures
Printed in Great Britain

A REFLEX INCREASE IN HEART RATE FROM DISTENSION OF THE PULMONARY-VEIN–ATRIAL JUNCTIONS

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(*Received* 26 *April* 1963)

Daly, Ludány, Todd & Verney (1937) reported that distension of the left atrium and pulmonary veins caused bradycardia and hypotension. Since then bradycardia has been assumed to be a characteristic response to distension of the left side of the heart (see reviews: Aviado & Schmidt, 1955; Heymans & Neil, 1958). Recently other investigators have failed to produce any changes in heart rate by distension of the left atrium alone and have attributed the bradycardia solely to distension of the left ventricle (e.g. Aviado & Schmidt, 1959). It has also been claimed that the increase in urine flow observed during distension of the left atrium in dogs was caused by a reflex decrease in secretion of antidiuretic hormone from the neurohypophysis (Gauer, Henry & Sieker, 1961), but this diuresis was shown not to result from a diminished release of antidiuretic hormone (Ledsome, Linden & O'Connor, 1961). Thus the response to distension of the left atrium, and by inference to stimulation of the so-called left atrial receptors, appears to be uncertain.

In dogs a histological examination (Nonidez, 1937) and a neurophysiological and histological study (Coleridge, Hemingway, Holmes & Linden, 1957) have shown that the majority of the receptors are situated in the subendocardial tissue at the junctions of the pulmonary veins and left atrium. The present investigation represents an attempt to distend only that part of the wall of the left atrium which contains the receptors. Distension of the pulmonary-vein–atrial junctions resulted in a reflex increase in heart rate and arterial blood pressure. The afferent path of the reflex was in the vagus nerves and the efferent path in the cardiac sympathetic nerves. A preliminary account of this investigation has been given (Ledsome & Linden, 1963*a*).

METHODS

Dogs of 12–25 kg were given a subcutaneous injection of morphine sulphate (0·5 mg/kg). One hour later under local anaesthesia a catheter was inserted through a saphenous vein into the inferior vena cava and each animal was anaesthetized by an intravenous infusion

of a solution of chloralose (British Drug Houses: dose 10 ml. = 0·1 g/kg) in sodium chloride solution (0·9 g/100 ml.). Subsequently during the experimental procedures a steady state of light anaesthesia was maintained by the infusion every 15 min of chloralose (1 g/100 ml.) about 1 ml./kg. As soon as possible after induction of anaesthesia artificial respiration was started with oxygen, humidified at room temperature and supplied from a Starling 'Ideal' pump, the rate (about 20/min) and stroke (about 50 ml./3 kg body wt.) of which were adjusted approximately to equal that of the animal's spontaneous respiration. When the chest was opened a resistance to expiration was provided by placing the expiratory outlet from the respiratory pump under 2–3 cm of water.

The left side of the chest was opened in the fifth intercostal space and the lung retracted laterally. The pulmonary veins were dissected free of their attachments and ligatures placed around three main pulmonary veins close to the lung. A small rubber balloon (5 mm long) coated with silicone ('Repelcote'; Hopkin & Williams) and attached to a nylon catheter (1 mm bore) was inserted into each pulmonary vein and tied so that its tip lay at the junction of the pulmonary vein with the left atrium. The pulmonary-vein–atrial junctions could then be distended by injecting into each balloon 0·5–1·5 ml. saline (NaCl 0·9 g/100 ml.) at 38° C. Soft strings were placed around the roots of the lobes of the left lung and tied immediately behind the balloon catheters thus occluding all structures within the left lung root. The balloon catheters were led out of the chest and clamped.

Pressures in the cardiovascular system were recorded through metal cannulae (Inconel; Johnson, Matthey & Co., London; 1·5 mm bore), treated with a solution of dialkyl dimethyl-ammonium chlorides ('Arquad'; Armour Hess, Ltd.) as a non-wetting agent, and inserted into the right femoral artery, the right atrium through the external jugular vein, a branch of the left pulmonary artery and the left atrium through the appendage. To each of the four cannulae was attached a Statham strain gauge (Model P 23 Gb) and after amplification by means of a carrier amplifier (S.E. Laboratories, Feltham, Middlesex) the pressure was recorded with a direct writing ultra-violet light recorder (S.E. Laboratories; Feltham, Middlesex). The frequency response of all systems obtained by the method of Linden (1959) was flat (±5%) to better than 60 c/s. Mean pressure was obtained electrically by passing the amplifier output through a simple R-C network with a time constant of 1 sec. In some experiments a similar system was used to record pressure inside the pulmonary vein balloons. The manometers were calibrated in a stepwise manner using mercury and saline manometers; zero pressure for each manometer was recorded post mortem as pressure at the cannula tip with the tip free in air.

In some experiments the right side of the chest was opened in the 4th intercostal space and a fine stainless-steel wire placed around the right vagus nerve immediately above the root of the right lung or around both roots of the right ansa subclavia. Similar wires were also placed around the left vagus nerve immediately above the root of the lung and at the level of the upper border of the aorta and around both roots of the left ansa subclavia. These nerves could then be cut easily, without disturbing the animal, by drawing the looped wire through a metal tube (1·5 mm bore) the end of which was sharpened.

During the surgical procedures, about 2 hr, the animals received a slow infusion of dog blood, plasma or dextran ('Dextraven', Benger Laboratories Ltd.) of approximately 8% of their estimated blood volume (1 l. for 13 kg body weight). Blood and plasma were used only in the preliminary experiments; the reasons for using dextran have been discussed elsewhere (Ledsome & Linden, 1963*b*). The electrocardiogram was recorded from leads on the forelegs or chest wall. The rectal temperature was maintained at 37·5° (±1°) C by adjusting heating lamps above and beneath the animal.

The pH and total carbon dioxide content of samples of arterial blood were measured; the methods used have been described previously (Ledsome & Linden, 1963*b*).

RESULTS

In twenty-four dogs when recording began about 2 hr after the initial anaesthetic was given the average heart rate was 100 beats/min (range 39–168; S.E. of mean ±7·6) and the average mean femoral arterial pressure was 131 mm Hg (range 98–160; S.E. of mean ±3·9). The average pH and carbon dioxide concentration in arterial blood fell within the limits previously described (Ledsome & Linden, 1963*b*).

As it was intended to examine a hypothetical reflex involving changes in heart rate it was important to establish that each animal showed known reflex changes in heart rate. Accordingly two reflex responses were tested; first, the carotid arteries were occluded, and secondly, the volume of gas in the lungs was increased by increasing the stroke of the respiratory pump. The reflex responses to these manoeuvres in this type of preparation have been described in detail elsewhere (Ledsome & Linden, 1963*b*); both manoeuvres evoke a response of an increase in heart rate, the first through both vagal and sympathetic pathways and the second through vagal pathways alone. The two reflexes were tested at intervals throughout each experiment and if one or other of these reflexes was unexpectedly absent the experiment was terminated and any results obtained after the previous positive test reflex response were rejected. For this reason two experiments were terminated, but only after more than 7 hr of anaesthesia.

Distension of the balloons in the pulmonary-vein–atrial junctions caused an increase in heart rate during each of 78 distensions in twenty-four dogs; the average of the increases in heart rate, the individual values of which are shown in Fig. 1, was 24 beats/min (range 2–89). A further analysis emphasizes the significance of the results; the average heart rate before distension of the balloons was 91 beats/min (range 36–168, S.E. of mean ±4·3), increased during distension to 119 beats/min (range 42–219, S.E. of mean ±5·6); 3 min after the release of the distension the average heart rate was 101 beats/min (range 37–186, S.E. of mean ±5). Distension of the balloons in the pulmonary-vein–atrial junctions also caused changes in mean femoral arterial pressure; the average change was an increase of 3·6 mm Hg (S.E. of mean ±0·7) and the individual changes in 78 distensions are also shown in Fig. 1. Mean femoral arterial pressure increased or did not change (range 0–25 mm Hg) in 65 distensions and decreased (range 0–20 mm Hg) in 13 distensions. The average mean arterial pressure before distension of the balloons was 127 mm Hg (range 97–162, S.E. of mean ±2), increased during distension to 131 mm Hg (range 81–170, S.E. of mean ±2·5) and then decreased after release of the distension to 127 mm Hg (range 100–169; S.E. of mean ±2·2). Examined by the technique of analysis of variance the pressures during balloon distension differ

significantly ($P < 0{\cdot}01$) from the pressures during the control periods before and after distension.

In Fig. 1 the changes in heart rate and mean arterial pressure in the individual experiments are shown together. There was no obvious relation between the changes in heart rate and in arterial pressure. In thirteen distensions in five dogs the systemic arterial pressure fell during distension of the balloons. Although a fall in blood pressure may well result from the

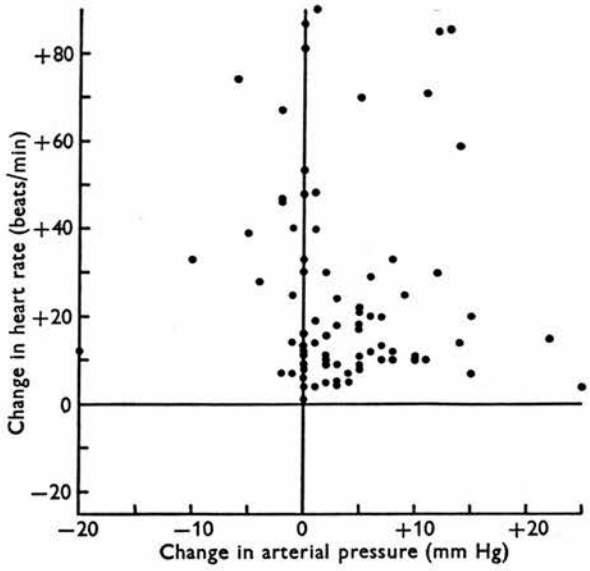

Fig. 1. Effects of distension of the pulmonary-vein–atrial junctions; 78 distensions in 24 dogs. 'Change in heart rate' is the heart rate in the third minute of distension minus the average of the heart rates 1 min before distension and in the third minute after release of the distension. 'Change in arterial pressure' is the femoral arterial mean pressure in the third minute of distension minus the average of the femoral arterial mean pressure 1 min before distension and in the third minute after release of the distension.

increase in heart rate, these dogs were not used in the further investigation of the reflex response to distension of the pulmonary veins because in them at least part of the response of an increase in heart rate may have been secondary to the fall in pressure in the systemic baroreceptor areas.

Because of the extensive range of response of the heart rate and blood pressure to distension of the pulmonary-vein–atrial junctions, three experiments have been selected to illustrate first a response typical of the average (Fig. 2) and then the extremes of the heart rate and arterial pressure changes (Figs. 3 and 4). Figure 2 is composed of sections of the experimental record taken before distension of the pulmonary-vein–atrial

junctions, during distension, and 1 min after the cessation of the distension. Heart rate increased by 22 beats/min during distension and although systolic pressure fell slightly diastolic pressure increased, so that mean arterial pressure increased by 5 mm Hg. At the same time there were similar changes in pulmonary arterial pressure and a small decrease (1 cm H_2O) in both left and right atrial pressures; the pattern of the

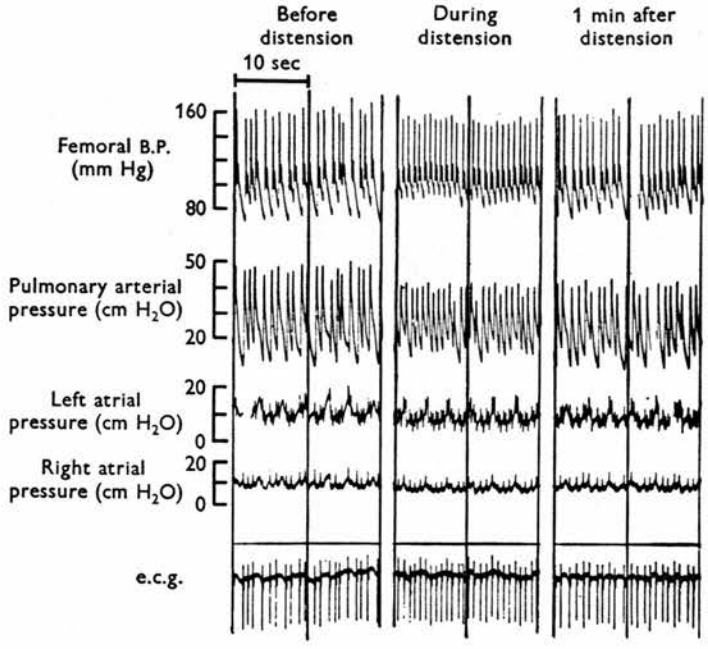

Fig. 2. Effects of distension of the pulmonary-vein–atrial junctions in dog 25; experiment illustrating a response typical of the average changes found. From above downwards femoral arterial pressure (mm Hg), pulmonary arterial pressure (cm H_2O), left atrial pressure (cm H_2O), right atrial pressure (cm H_2O), datum line and electrocardiogram. First column recorded immediately before distending balloons in the pulmonary veins, second column after the balloons had been distended for 3 min, third column 1 min after removal of the distension.

electrocardiogram was unaltered. Figure 3 shows records from an experiment in which there was a large increase in heart rate with little or no change in mean arterial pressure. In this experiment heart rate increased from 80 beats/min before distension of the balloons to 170 beats/min during distension and decreased to 105 beats/min at 3 min after stopping the distension. Systolic arterial pressure decreased but mean arterial pressure remained unchanged. Pulmonary arterial pressure again showed changes similar to the changes in systemic pressure, and left atrial pressure decreased by 4 cm H_2O and right atrial pressure by 2·5 cm H_2O. The

record in Fig. 4 illustrates an experiment in which there was an increase in arterial pressure but little change in heart rate. The initial heart rate in this experiment was exceptionally slow, 36 beats/min, increased to 44 beats/min during distension and then decreased to 40 beats/min at 3 min after distension. Both systolic and diastolic pressures increased, so that mean arterial pressure was increased by 15 mm Hg. In this experiment there was no change in pulmonary arterial pressure or left atrial pressure. Although the type of response varied from dog to dog it was

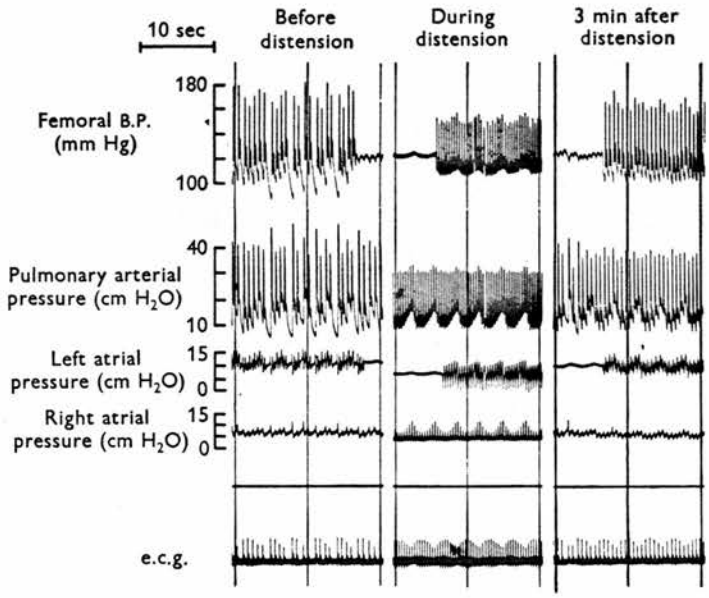

Fig. 3. Effects of distension of the pulmonary-vein–atrial junctions in dog 26; record of an experiment in which there was a large increase in heart rate. Conventions as in Fig. 2. Parts of femoral arterial record and left atrial record show mean pressures.

usual to find that in any one animal repeated distensions of the balloons elicited similar responses. However, that the differences in response may depend in some way on the difference in initial heart rate, possibly related to differences in the background of vagal and/or sympathetic tone, is suggested by the following experiment. In the dog from which the record illustrated in Fig. 4 was obtained the response was altered by bleeding 100 ml. of blood from the animal. This haemorrhage resulted in a fall in mean arterial pressure of 30 mm Hg and an increase in the control heart rate to 96 beats/min. When after this the pulmonary-vein balloons were distended, heart rate increased to 130 beats/min and mean arterial pressure increased only 5 mm Hg.

The changes in heart rate and arterial pressure began within 15 sec of the onset of distension of the pulmonary-vein–atrial junctions and a steady state was reached within 3 min. After the distension was released the heart rate and arterial pressure did not return to the pre-distension values immediately but declined gradually during 30 sec to 3 min. In two dogs in which the responses were large it was 6 min and 9 min before heart rate returned to the pre-distension values. In most of the experiments the

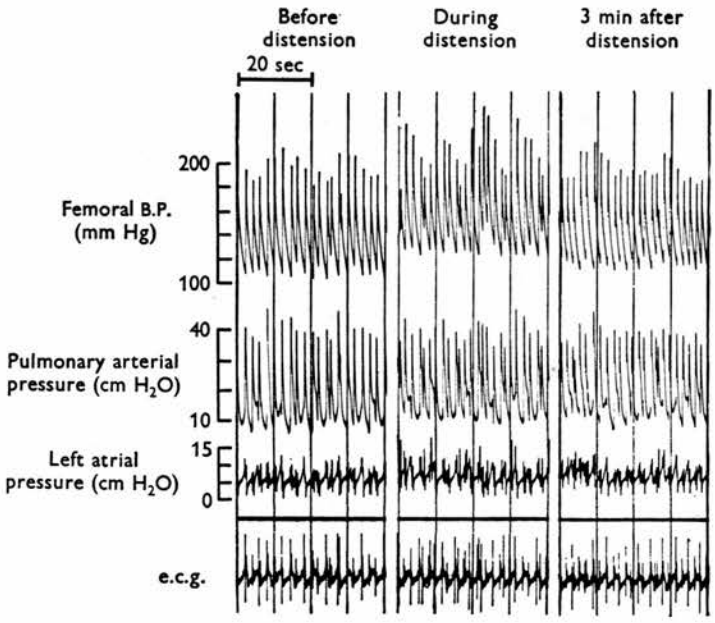

Fig. 4. Effects of distension of the pulmonary-vein–atrial junctions in dog 13; record of an experiment in which there was an increase in femoral arterial pressure. Conventions as in Fig. 2.

balloons were distended only for 3 min, but in eight experiments distension was maintained for 30 min and during each of these distensions the changes in heart rate and arterial pressure were maintained throughout the period of distension.

The balloons were so designed that with the volumes of saline injected (0·5–1·5 ml.) the pressure within the balloons would be very high (about 200 mm Hg) compared with that in the atria (about 10 mm Hg) and surrounding tissue; thus each balloon would attain a spherical shape of predetermined size. However, although the object of the authors was to distend to a predetermined volume so as to stretch the pulmonary-vein–atrial junctions (see Discussion) it may be suggested that excessive force was used to distend the tissues. Therefore in four dogs the pressure within

each balloon was measured at each distension volume under two conditions; first, during the experimental periods, during which the heart rate response was observed, and secondly, when the balloons were free in air post mortem. The pressure within the balloon gives no indication of the degree of stretch imposed on the tissues; but an indication of the 'stretching force' may be obtained from a consideration of the difference in pressure within the balloon in the two positions (pressure measured in the balloon during the experimental distension *in vivo* minus the pressure in the balloon free in air post mortem). In 29 balloon distensions in the four dogs the mean difference in the two pressures was 24 mm Hg (range 6–48; S.E. of mean $\pm 2{\cdot}2$); in 15 of the 29 distensions the 'stretching force' was below 15 mm Hg and in 4 of these it was below 10 mm Hg. During all these experimental distensions the heart rate increased. No relation between the difference in pressure (the so-called 'stretching force') and the increase in heart rate was observed.

In some experiments as the volume of the balloons was increased from 0·5 to 1·5 ml. the 'stretching force' decreased in value. For instance, in one dog the balloons were distended successively with 0·5 and 1·5 ml. saline; the increase in heart rate with the distensions was 10 and 21 beats/min respectively, but the three balloons showed differences in pressure ('stretching force') of 10, 15 and 29 mm Hg during distension with 0·5 ml. saline but 7, 8 and 16 mm Hg during distension with 1·5 ml. saline. Thus it is probable that with this small balloon in this situation the difference between the pressure within the balloon *in vivo* and post mortem does not give a reasonable measure of the force which stretches the surrounding tissues. But the degree of stretch *is* indicated by the volume of fluid injected into the balloons.

Further experiments were carried out in an attempt to define afferent and efferent pathways and so establish the reflex nature of the response of an increase in heart rate and blood pressure. Afferent nerve fibres from many intrathoracic receptors have been described in the cervical vagus nerves and therefore the effects were examined of cutting or cooling (to 6° C) the vagus nerves at different levels in the neck and thorax (see Table 1). In the first dog (No. 7, Table 1) the effects of distension of the pulmonary-vein balloons (increase in heart rate and blood pressure) were abolished by cutting the left vagus nerve in the neck. In the next dog (No. 9, Table 1) the left vagus nerve was cooled in the neck; there was no increase in heart rate on distension of the balloons whilst the nerve was cooled, but the effect was present after the nerve was warmed. Next, the effects of cutting the vagus nerves caudal to the origin of the efferent cardiac branches were examined. In dog No. 11 (Table 1), therefore, the left vagus nerve was cut immediately above the root of the lung; this

TABLE 1. The effect of distension of the pulmonary-vein–atrial junctions on heart rate and mean arterial pressure before and after section or cooling of the vagus nerves at different levels in the thorax and neck

Dog no.	Increase in heart rate and mean blood pressure											
	Control distension		After section of vagus in thorax at level of:						After section of vagus in neck			
			L. lung root		R. lung root		L. upper border of aorta		Left		Right	
	(beats/min)	(mm Hg)	(beats/min)	(mm Hg)	(beats/min)	(mm Hg)	(beats/min)	(mm Hg)	(beats/min)	(mm Hg)	(beats/min)	(mm Hg)
7	14	8	—	—	—	—	—	—	0	0	—	—
9	10	0	—	—	—	—	—	—	*0	0	—	—
11	70	15	19	18	—	—	—	—	*10	12	*0	0
24	30	9	7	0	2	0	—	—	—	—	—	—
25	13	6	10	5	9	0	8	0	0	0	—	—
26	77	0	66	0	22	0	3	0	—	—	—	—

* After cooling vagus to 6°C (thermode to 5°C).

caused a dramatic reduction in the effect of distension of the pulmonary-vein–atrial junctions. There was a small further reduction when the left vagus nerve was cooled in the neck and when this nerve was warmed and the right cervical vagus nerve was cooled the response was abolished. The last three dogs were treated similarly (Nos. 24, 25, 26, Table 1). First, the left vagus nerve was cut immediately above the root of the lung; this

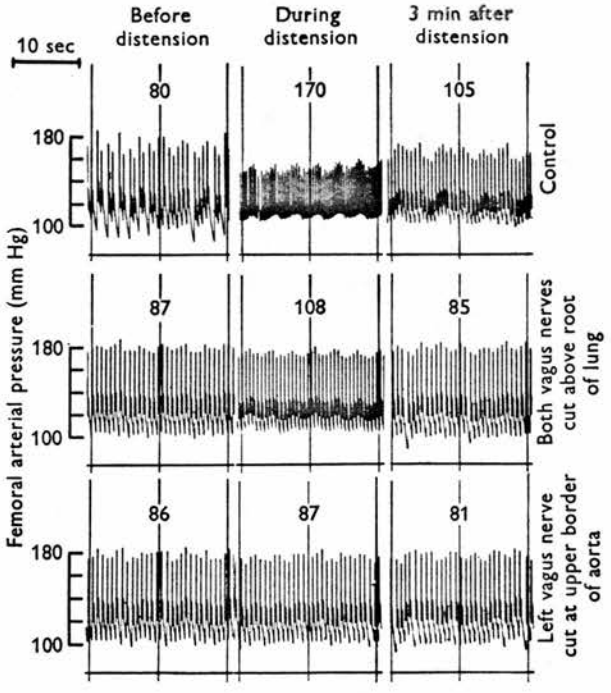

Fig. 5. The effect of cutting the vagus nerves on the response to distension of the pulmonary-vein–atrial junctions in dog 26. Consecutive parts of the femoral arterial pressure record in one experiment. Records taken before distension, during distension (after 3 min) and 3 min after removing the distension of the balloons. Upper line control, middle line after cutting both vagus nerves immediately above the lung roots, lower line after cutting the left vagus nerve at the level of the upper border of the aorta. Numerals above traces show heart rates (beats/min).

reduced the response to distension of the balloons. Next, the right vagus nerve was cut at the level of the upper border of the root of the lung; this abolished the response in one dog (No. 24, Table 1) and reduced the response in another (No. 26, Table 1), illustrated in Fig. 5. The left vagus nerve was then cut at the level of the upper border of the aorta; this abolished the response in the experiment shown in Fig. 5 (No. 26, Table 1) but had no further effect in the remaining dog. The response to distension

of the balloons in this remaining dog (No. 25, Table 1) was abolished only when the left vagus nerve was cut in the neck. After abolition of the effects of distension of the balloons in these dogs an intact efferent vagal pathway to the heart was always demonstrated; there was an immediate slowing of the heart on release of carotid occlusion, and increased inflation of the lung (except in the experiments in which the right vagus nerve was cut) caused an increase in heart rate. An example of such a test is shown

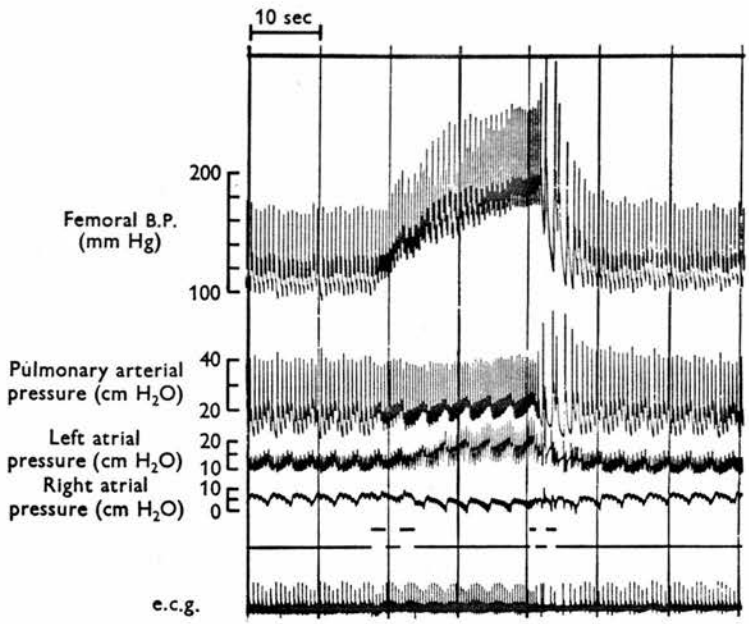

Fig. 6. The effect of occluding both carotid arteries immediately after the last record shown in Fig. 5. Record demonstrates an intact efferent vagal pathway to the heart. Conventions as in Fig. 2. The first two signal marks indicate times of occluding the carotid arteries and the second two signal marks indicate release of the occlusion.

in Fig. 6; this shows the effect of carotid occlusion performed immediately after the last record in Fig. 5, there is marked 'vagal slowing' on release of this occlusion. The results were not affected by the prior cutting of the vagus nerves caudal to the lung roots in two dogs (Nos. 9, 11, Table 1). Thus the response to distension of the pulmonary-vein–atrial junctions was first reduced and then abolished by cutting afferent fibres in the vagus nerves.

The gradual onset and decline of the response and the range of variation in the changes in heart rate and blood pressure closely resembled the effects of stimulating the cardiac sympathetic nerves in this type of preparation (Ledsome & Linden, 1963*b*). The effects of interrupting the

sympathetic nerves to the heart on the response to pulmonary vein distension were therefore examined in five dogs (Table 2). In four dogs both roots of the left and then the right ansa subclavia were cut. The increase in heart rate and arterial pressure caused by distension of the pulmonary-vein–atrial junctions was always present when one ansa sub-

TABLE 2. The effect of distension of the pulmonary-vein–atrial junctions on the heart rate before and after section of both ansae subclaviae and/or the injection of bretylium tosylate. Experiments 21 and 22 showed a rise in mean arterial blood pressure of 8 mm Hg and 5 mm Hg during the control distension; these responses were not present after injection of bretylium tosylate in No. 21 and after section of the ansae subclaviae in No. 22

	Increase in heart rate (beats/min)		
Dog no.	Control distension	After section of both ansae subclaviae	After injection of bretylium tosylate (10 mg/kg)
12	17	0	Not given
21	48	16	0
22	18	1	Not given
29	53	Not sectioned	7
34	18	4	0

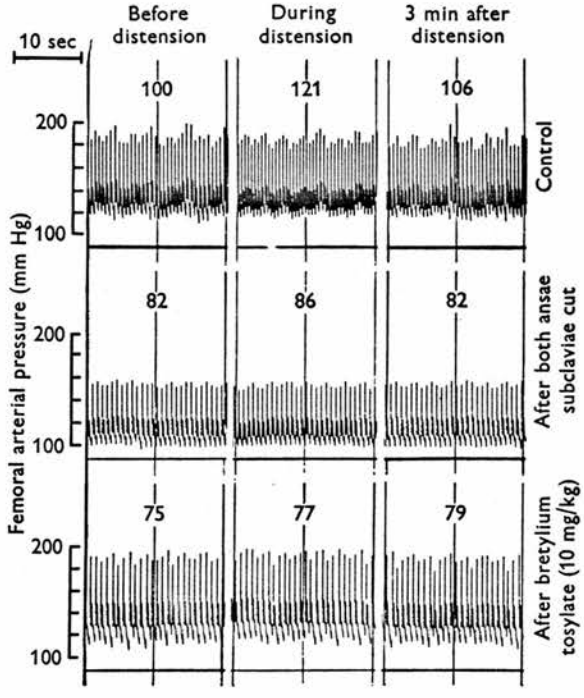

Fig. 7. The effect of preventing the action of the cardiac sympathetic nerves on the response to distension of the pulmonary-vein–atrial junctions in dog 34. Conventions as in Fig. 5. Upper line control, middle line after cutting both ansae subclaviae, lower line after injecting bretylium tosylate 10 mg/kg.

clavia was intact. After both ansae subclaviae had been cut the response was completely abolished in two dogs (Nos. 12 and 22, Table 2), almost abolished in one dog (No. 34, Table 2 and Fig. 7) and reduced in the fourth dog (No. 21, Table 2). The latter two dogs were given bretylium tosylate ('Darenthin'; Burroughs Wellcome & Co.) 10 mg/kg which completely abolished the remaining effects of balloon distension. In a fifth dog (No. 29, Table 2) in which both ansae subclaviae were intact the response was almost completely abolished by the injection of bretylium tosylate 10 mg/kg. After cutting both ansae subclaviae and injecting bretylium tosylate there was still an increase in heart rate in response to carotid occlusion and to increased inflation of the lung. These results strongly suggest that the effects of distending the pulmonary-vein–atrial junctions were produced reflexly and that the efferent path of the reflex was in the cardiac sympathetic nerves.

DISCUSSION

Many attempts have been made to relate changes of pressure in, and distension of, the chambers of the heart and large vessels to changes in heart rate and blood pressure (see p. 456). It has been shown that intravenous infusions of blood or saline may cause an increase in the heart rate of both anaesthetized and unanaesthetized dogs (Bainbridge, 1915; Anrep & Segall, 1926; Ballin & Katz, 1941; Coleridge & Linden, 1955; Jones, 1962). This effect was attributed by Bainbridge (1915) to a reflex, the effective stimulus for which was an increase in right atrial pressure. Evidence for this claim is lacking; distension of the right atrium and venae cavae in some experiments had no effect on heart rate (Ballin & Katz, 1941) and in other experiments a rise in right atrial pressure caused bradycardia (Aviado, Li, Kalow, Schmidt, Turnbull, Peskin, Hess & Weiss, 1951); also any increase in heart rate resulting from infusion was attributed to chemoreceptor activity (Aviado & Schmidt, 1955).

Although a bradycardia has been observed to result from a rise in pressure or distension of the left side of the heart (Daly & Verney, 1927; Daly *et al.* 1937; Aviado & Schmidt, 1955, 1959) this response has been attributed to distension of the left ventricle; there was no change in heart rate when the left atrium alone was distended (Aviado & Schmidt, 1959). No response to distortion of the heart walls or great veins was observed by Klussman, Van Citters & Rushmer (1960).

Thus attempts to stimulate receptors in the large veins or in the chambers of the heart, particularly in the left side of the heart, have resulted either in no change in heart rate or a bradycardia. In contrast, in the present investigation the heart rate always increased when the pulmonary-vein–atrial junctions were distended (Fig. 1). Although it is not profitable

to attempt to explain the results of other investigations, there are important differences between this and previous investigations. In this investigation large-scale perfusion systems were avoided and care was taken to mitigate the metabolic acidosis known to occur in anaesthetized, surgically traumatized animals (Millar & Morris, 1961) and to ensure an adequate supply of oxygen to the tissues. Animals prepared in this way had active cardiovascular reflexes with functioning efferent pathways in both the vagus and sympathetic nerves (Ledsome & Linden, 1963*b*). Also although the stimulus was crude only that part of the wall of the atrio-venous junctions known to contain the atrial receptors was distended.

The experiments described in this paper show that distension of the junctional regions of the pulmonary veins and left atrium causes an increase in heart rate and an increase in mean arterial pressure. The response could not have resulted from interference with the blood flow to or from the left lung because the left lung root was tied off and no blood was flowing through it. The balloons were small and caused no obstruction to flow to or from the atrium.

The response to pulmonary vein distension was abolished by cutting the vagus nerves in the neck or by cutting the vagus nerves in the thorax caudal to the branching of the efferent cardiac nerves. A functioning vagal efferent pathway to the heart was demonstrated in each experiment after the response to pulmonary vein distension had been abolished (e.g. Fig. 6); the response was therefore abolished by cutting afferent fibres in the vagus nerves. In these experiments no attempt was made to define the particular branches of the vagus nerves which contained these afferent fibres and indeed it appears from the limited evidence the results provide that the precise path varied from dog to dog. The results do, however, demonstrate that the afferent path of the reflex lies in the vagus nerves and that at least some of the fibres are to be found in the cervical vagus nerves.

The gradual onset and slow decline of the response to distension of the pulmonary-vein–atrial junctions and the increase in mean arterial pressure closely resembled the effects produced by stimulation of the ansa subclavia in dogs with the vagus nerves intact (Ledsome & Linden, 1963*b*). Most if not all of the sympathetic accelerator fibres to the heart are to be found in the ansae subclaviae (Mizeres, 1958) and bretylium tosylate 10 mg/kg in this type of preparation blocks post-ganglionic sympathetic nerves without affecting afferent nerves, heart rate changes mediated through efferent vagus nerves (Ledsome & Linden, 1963*b*) or effects produced by increased release of catecholamines from the adrenal medulla (Boura & Green, 1959). The increase in heart rate and mean arterial pressure caused by distension of the pulmonary-vein–atrial junctions was abolished either

by cutting both ansae subclaviae or by injection of bretylium tosylate 10 mg/kg. It was therefore concluded that the effects of distension of the pulmonary-vein-atrial junctions were the result of increased activity in efferent sympathetic nerves to the heart, and there was no evidence of any effect through efferent vagal pathways or through other sympathetic pathways.

Inflation of the lung causes a reflex increase in heart rate (Anrep, Pascual & Rossler, 1936; Daly & Scott, 1958; Ledsome & Linden, 1963*b*); both afferent and efferent pathways of the reflex are in the vagus nerves. That the response to pulmonary vein distension is independent of the lung inflation reflex is suggested by two facts: first, that with both lungs denervated (lung inflation reflex abolished, Ledsome & Linden, 1963*b*) distension of the pulmonary-vein-atrial junctions still resulted in a reflex increase in heart rate (Fig. 5); secondly, the efferent pathway involved in the reflex response to distension of the pulmonary veins lies solely in the sympathetic nerves, whereas an efferent sympathetic limb to the lung inflation reflex could not be demonstrated (Ledsome & Linden, 1963*b*).

We do not claim to have proved that the receptors involved in this reflex are those in the walls of the left atrium; but they are the receptors most likely to have been stimulated. Histological investigations (Nonidez, 1937, 1941) and combined electrophysiological and histological examinations in the dog (Coleridge *et al.* 1957) have shown the so-called atrial receptors to be situated in the subendocardial tissue at the junctions of the veins and atria. The afferent fibres from these receptors are in the cervical vagus nerves. The adequate stimulus to the atrial receptors is unknown. Two types of atrial receptors have been proposed by Paintal (1953): Type A, which increased their discharge during atrial systole and showed variable bursts of activity in time with the atrial filling phase, and which were said to respond to changes in pressure, and Type B receptors, said to discharge only during the atrial filling phase and to respond to atrial distension. Infusion of fluid to increase atrial filling results in an increased discharge from Type B receptors (Coleridge *et al.* 1957) and there is a linear relation between their discharge and atrial pressure, during infusion and drug-induced bradycardia (Kramer, 1959). However, Pearce, Henry & Chapman (1956) have found that Type B receptors could be caused to discharge during atrial contraction after severe haemorrhage and during increased intrathoracic pressure or the infusion of adrenaline. Although the hypothesis most likely to be true is that there is one type of receptor and the adequate stimulus is probably a rate of change of deformation, it seemed from the evidence that an effective stimulus would be an increase in the area, i.e. a stretch of the wall of that part of the atrio-venous

junction known to contain the receptors. Therefore balloons were made which required a large force to distend them, so that regardless of the pressure in the atrium they would occupy a spherical shape and the volume and circumference would be known. An attempt was made to obtain an indication of the force with which the distended balloons stretched the wall of the atrio-venous junctions. But the difference between the pressure in the balloons *in vivo* and free post mortem (the 'stretching force') gave little indication of the degree of stretching as indicated by the volume of fluid in the balloon. However, responses of an increase in heart rate and blood pressure were obtained in experiments in which the pressure difference ('stretching force') was within the range of pressures observed in the intact left atrium, indicating that on these occasions, at least, no excessive force was used to distend the tissues. It is concluded that even with this crude method of stimulation the receptors most likely to be stimulated and to be involved in the reflex described in this investigation are those situated at the junction of the left atrium and pulmonary veins—the left atrial receptors.

It is possible that some of the afferent fibres involved in the reflex response from the pulmonary veins traverse the thoracic sympathetic system. Nonidez (1941) observed no receptor endings in the pulmonary veins of three previously sympathectomized cats. On the basis of this evidence he suggested that afferent nerve fibres from pulmonary veins traverse the thoracic sympathetic system. It is not known whether these fibres enter the spinal cord or rejoin the vagus nerves. Again, the possibility cannot be excluded that other nerve endings in the mediastinum around the left lung root may be affected by the stimulus. Holmes & Torrance (1959) have described impulse activity in afferent fibres in the ansae subclaviae of the cat arising from receptors situated in the mediastinum. The function of these receptors is unknown. However, the response to distension of the pulmonary veins cannot result solely from an increase in activity of afferent sympathetic fibres which enter the spinal cord because the response to pulmonary vein distension could be abolished by cutting one or other vagus nerve in the neck.

Because stimulation of the stellate ganglion always results in an increase in contractility of heart muscle (Linden, 1963) it is probable that this reflex response of an increase in heart rate would be accompanied by an increase in contractility of atrial and ventricular muscle which would result in an increased cardiac output and lowered mean atrial pressure. The increased blood pressure seen in some of the responses in this investigation may well be the result of such a mechanism. This reflex may thus provide a second means by which the heart could adjust the output to the input—the first being the Starling mechanism (Starling, 1918).

SUMMARY

1. Distension of small balloons in the pulmonary-vein–atrial junctions always caused an increase in heart rate and usually an increase in mean arterial pressure.

2. These effects of distension of the junctional regions of the pulmonary veins were produced reflexly.

3. The afferent path of the reflex was in the vagus nerves and the efferent path in the cardiac sympathetic nerves.

4. The receptors most likely to be stimulated by distension of the pulmonary-vein–atrial junctions are those receptors situated in the endocardium of this part of the left atrium.

The authors are grateful for the technical assistance of Mr G. Wade and Mr J. Brook. This investigation was supported by Public Health Service Research Grant No. H. 6106, from the National Heart Institute, U.S.A.

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J. Physiol. (1967), **193**, *pp.* 121–129
With 3 text-figures
Printed in Great Britain

THE EFFECT OF DISTENDING A POUCH OF THE LEFT ATRIUM ON THE HEART RATE

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(*Received* 5 *May* 1967)

SUMMARY

1. A pouch has been prepared consisting of a part of the wall of the left atrium together with the left pulmonary vein–atrial junctions.

2. An increase of perfusion pressure in the pouch caused a reflex increase in heart rate.

3. The afferent path of the reflex was in the vagus nerves and the efferent path was in the sympathetic nerves.

4. The receptors most likely to be stimulated by an increase in pressure in the pouch are those receptors situated in the subendocardium of the pulmonary vein–left atrial junctions.

INTRODUCTION

It has been shown (Ledsome & Linden, 1964*b*) that distension of small balloons in the pulmonary vein-atrial junctions of anaesthetized dogs causes a reflex increase in heart rate. The receptors most likely to be stimulated by distension of the pulmonary vein–atrial junctions are those receptors situated in the endocardium of this part of the left atrium (Coleridge, Hemingway, Holmes & Linden, 1957). Because of the difficulty in assessing the force used to distend the tissues by means of balloons, an isolated pouch consisting of a part of the left atrium together with the pulmonary vein–atrial junctions has been prepared and perfused. An increase in perfusion pressure in the pouch caused a reflex increase in heart rate qualitatively similar to that previously described (Ledsome & Linden, 1964*b*). The afferent path of the reflex was in the vagus nerves and the efferent path was in the cardiac sympathetic nerves.

METHODS

Dogs of 13–20 kg were given a subcutaneous injection of morphine sulphate (0·5 mg/kg). One hour later under local anaesthesia (decicain, 2%) a catheter was inserted through a saphenous vein into the inferior vena cava of each animal; seven dogs were anaesthetized by an intravenous infusion of a solution of chloralose (British Drug Houses: dose

10 ml. = 0·1 g/kg) in sodium chloride (0·9 g/100 ml.) and in three dogs pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd: dose 20 mg/kg) was injected. Subsequently during the experimental procedures a steady state of light anaesthesia was maintained by the infusion every 15 min of chloralose (dose about 1 ml. = 0·01 g/kg) or by the injection every ½ hr of pentobarbitone sodium, about 1 mg/kg. As soon as possible after induction of anaesthesia artificial respiration was started with oxygen (100 % in six dogs, 40 % in four dogs), humidified at room temperature and supplied from a Starling 'Ideal' pump, the rate and stroke of which were adjusted approximately to equal that of the animal's spontaneous respiration. When the chest was opened a resistance, equivalent to 3 cm H_2O, was placed in the expiratory outlet of the respiratory pump.

The left side of the chest was opened in the fifth intercostal space and the lung retracted laterally. The pulmonary veins were dissected free of their attachments and ligatures placed around the three main left pulmonary veins close to the lung. A cannula directed towards the atrium was inserted into each of the three pulmonary veins. Later in the experiment the cannula in the pulmonary vein to the middle lobe was used to record pressure in the pouch and the other two cannulae acted as inlet and outlet channels from a Dale-Schuster pump. Two large clamps were placed at the root of the left lung lateral to the entry of the cannula nto the pulmonary veins; no blood or air could enter the left lung and the cannulae remained connected to the left atrium. With the left lung retracted to the left, the apex of the heart was lifted and gently pulled over to the right; under direct vision the *right* pulmonary veins were dissected free from the oesophagus behind. Care was taken not to damage the connective tissue adjoining the left pulmonary vein-atrial junctions. The dissection was carried upwards almost to the main pulmonary artery.

A stainless-steel clamp was made with two thin blades opposed and at right angles to the handles. The blades were 8 cm long and had a convexity to the right (radius of curvature 9 cm). The posterior blade of the clamp was introduced up the dissected channel behind the left atrium with the anterior blade resting on the anterior wall of the left atrium lateral to the atrial appendage. When closed, the tip of the clamp lay posterior to the pulmonary artery almost in contact with the arch of the aorta; the posterior blade rested solely on the oesophagus behind, and in front was applied to the wall of the left atrium at the left edge of the entrance of the right pulmonary veins into the left atrium. The atrium was thus clamped so as to form two chambers; one chamber into which the right pulmonary veins opened and which acted as a channel to the left ventricle; the second chamber which was a pouch made up of the left part of the left atrium, the left pulmonary vein–atrial junctions and the three pulmonary veins each containing a cannula. A record of systemic blood pressure was observed throughout the insertion of the clamp and the fact that systemic arterial blood pressure did not fall was taken to mean that the flow through to the left ventricle was not significantly obstructed. The pouch was immediately perfused with Ringer–Locke solution by means of a Dale-Schuster pump. The solution was kept at 38° C (± 1° C) and a mixture of 5 % CO_2 in O_2 continuously bubbled through it; heparin (Pularin, Evans Medical Ltd.) was added to give a concentration in the solution of 5000 i.u./l.

Pressures in the cardiovascular system were recorded through metal cannulae (Inconel; Johnson, Matthey and Co., London, 1·5 mm bore) treated with dialkyl dimethylammonium chlorides (Arquad, Armour Hess Ltd.) as a non-wetting agent, and inserted into the right femoral artery, the main body of the left atrium through the appendage and the isolated pouch of the left atrium through the middle left pulmonary vein. To each of the three cannulae was attached a Statham strain-gauge (model P 23 Gb) and after amplification by means of a carrier amplifier (S.E. Laboratories, Feltham, Middlesex) the pressure was recorded with a direct writing ultraviolet light recorder (S.E. Laboratories, Feltham, Middlesex). The frequency response of all systems obtained by the method of Linden (1959) was flat (± 5 %) to better than 60 c/s. Mean pressure was obtained electrically by passing the amplifier output through a simple R-C network with a time constant of 1 sec. The manometers were calibrated in a stepwise manner using mercury and saline manometers. Zero

pressure for each manometer was recorded post mortem as pressure at the cannula tip with the tip free in air.

During the surgical procedures, which lasted about 2 hr, the animals received a slow infusion of dextran (Dextraven, Benger Laboratories Ltd.) of approximately 8 % of their estimated blood volume (1·0 l. for each 13 kg body wt.). The electrocardiogram was recorded from leads attached to the forelegs or chest wall. Heart rate was recorded by means of a cardiotachometer (McCook & Peiss, 1959) triggered by the arterial pressure pulse; the output filter had a time constant of 8 sec. All heart rates included in the results were obtained by counting the QRS complexes of the electrocardiogram over at least 30 sec. The rectal temperature was maintained at 37·5° (± 1° C) by adjusting heating lamps above and beneath the animal.

The pH of arterial blood was measured in blood withdrawn anaerobically from a catheter in the left femoral artery with the tip in the abdominal aorta. Samples of 7 ml. of blood were drawn into warmed syringes in which the dead space was filled with heparin (Pularin: Evans Medical Ltd., 1000 i.u./ml. of 0·9 % sodium chloride). The blood was transferred immediately to electrode systems for measuring the P_{O_2}, P_{CO_2}, pH and bicarbonate concentration in the blood. Methods for the determination of these parameters have been described previously (Norman, Ledsome & Linden, 1965; Linden, Ledsome & Norman, 1965).

RESULTS

In ten dogs when recording began about 2 hr after the initial dose of anaesthetic the pH of the arterial blood was within the range 7·28–7·41; the P_{CO_2} was within the range 37–43 mm Hg; the concentration of bicarbonate in the plasma was within the range 18·5–22·2 mM; the P_{O_2} was greater than 100 mm Hg.

Distension of the left atrial pouch by means of pulsatile pressure caused an increase in heart rate during each of thirty-three distensions in ten dogs; the average increase in heart rate was 10·3 beats/min (range 3–27) from an average initial heart rate of 137 beats/min (range 88–239). In twenty-two of the distensions there was no change in femoral arterial mean pressure; in eleven distensions in five dogs there was an increase in mean femoral arterial pressure, average increase 4·5 mm Hg (range 2–10). There were no changes in pressure in the main body of the left atrium during any distension. Similar responses were seen in dogs anaesthetized with either chloralose or pentobarbitone sodium.

An example of the record obtained in one experiment is shown in Fig. 1. The heart rate during distension of the left atrial pouch was 17 beats/min faster than the heart rate either before or after the distension. Although the arterial pulse pressure decreased when the heart rate increased there was no change in mean arterial pressure. In this experiment the left atrial pouch was distended with a pressure of 43 cm H_2O on which was imposed an oscillation of about 20 cm H_2O in amplitude.

The changes in heart rate and blood pressure were calculated as the heart rate or blood pressure in the 3rd min of distension minus the average of the heart rates or blood pressures 1 min before distension and in the

3rd min after stopping the distension. The changes in heart rate began within 5–10 sec of distending the pouch and a steady heart rate was reached in about 30 sec. After the distension was stopped the heart rate fell gradually over 10–30 sec. The pressure in the pouch of the left atrium ranged from 0 to 5 cm H_2O during the control periods; superimposed upon the mean pressure during the control periods was an oscillation of 2–5 cm H_2O amplitude produced by contraction of the atrial wall. The rate of perfusion during the control periods was of the order of 50 ml./min. The

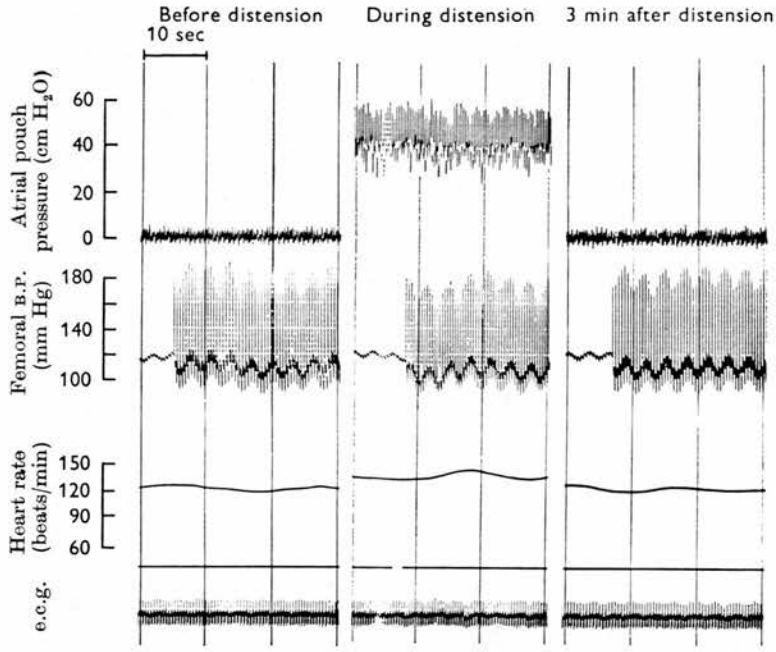

Fig. 1. Dog 35/64. Effects of distension of the left atrial pouch. From above downwards pressure in the left atrial pouch (cm H_2O), femoral arterial pressure (mm Hg), heart rate (beats/min), datum line and electrocardiogram. First column recorded 1 min before increasing perfusion pressure in the left atrial pouch, second column 3 min after increasing pressure, third column 3 min after pressure had been reduced.

average increase in mean pressure during pulsatile distension of the pouch was 31 cm H_2O (range 13–72); superimposed upon this mean pressure was an oscillation of amplitude 5–20 cm H_2O at a rate of 80–150/min determined by the rate (chosen arbitrarily) and stroke of the Dale-Schuster pump. To obtain this type of pulsatile distension the rate and stroke of the pump were increased and the outflow from the pouch was restricted. The rate of perfusion at this time was of the order of 1 l./min. The response of

the increase in heart rate was too small to allow any determination of a quantitative relationship between graded increases in pouch pressure and the increases in heart rate.

Because this response was considered to be mediated by the same reflex as that described previously (Ledsome & Linden, 1964*b*) further experiments were carried out in an attempt to define the afferent and efferent pathways. In three dogs the left vagus nerve was sectioned at the upper border of the aorta; in two dogs the response of the increase in heart rate was abolished and in one dog much reduced. The response was abolished

TABLE 1. The effects of distension of a pouch of the left atrium on the heart rate before and after cutting the vagus nerves. The figures in parentheses are the control heart rates for each distension

	Increase in heart rate (beats/min)		
		After section of vagus	
Dog. no.	Control	L. at upper border aorta	R. in neck
73/63	10 (137)	0 (153)	—
28/64	11 (166)	4 (163)	0 (172)
36/64	9 (139)	0 (138)	—

TABLE 2. The effects of distension of a pouch of the left atrium on the heart rate before and after an injection of sympathetic blocking agents. The numbers in parentheses are the control heart rates for each distension

	Increase in heart rate (beats/min)		
Dog no.	Control	After bretylium tosylate, 10 mg/kg	After propranolol 0·5 mg/kg
22/64	9 (88)	0 (78)	—
23/64	5 (110)	0 (78)	—
25/64	13 (113)	0 (136)	—
34/64	8 (236)	—	0 (167)
35/64	20 (135)	—	2 (94)

in the third dog after section of the right vagus nerve in the neck (see Table 1).

In five dogs the response to distension of the pouch of the left atrium of an increase in heart rate was abolished by the prior injection of a sympathetic nerve blocking agent; bretylium tosylate ('Darenthin'; Burroughs Wellcome and Co.), 10 mg/kg, was given in three dogs and propranolol ('Inderal'; I.C.I.), 0·5 mg/kg, in two dogs (see Table 2).

In Fig. 2 is a record obtained from the same dog as illustrated in Fig. 1 taken 5 min after the injection of propranolol 0·5 mg/kg; after injection of propranolol the heart rate was slower. Distension of the left atrial pouch after propranolol using the same increase in pressure in the pouch as before resulted in a change in heart rate of only 2 beats/min; the mean arterial pressure and pulse pressure were unchanged.

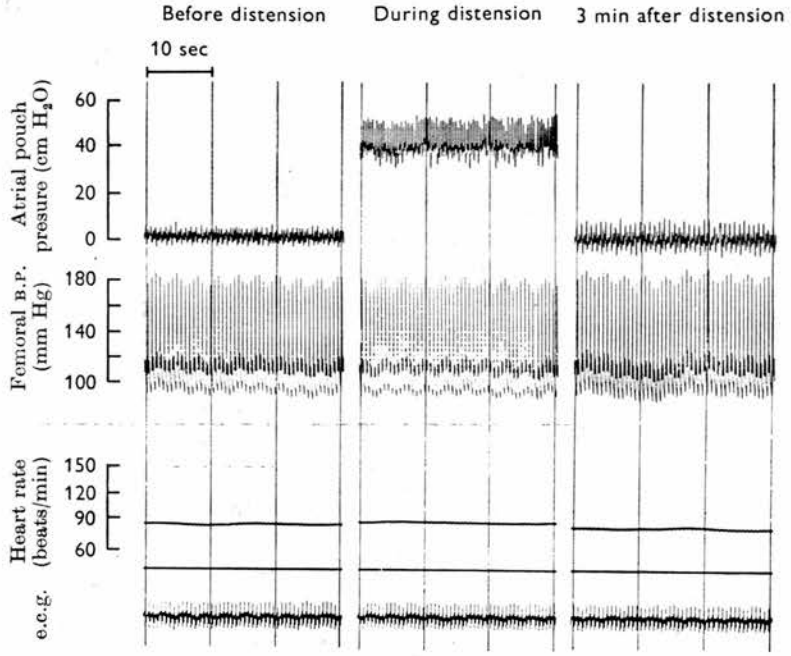

Fig. 2. Dog 35/64. Effects of distension of the left atrial pouch 5 min after the injection of propranolol 0·5 mg/kg. Conventions as in Fig. 1.

DISCUSSION

Ledsome & Linden (1964*b*) reported that distension of small balloons in the pulmonary vein–atrial junctions of the anaesthetized dog resulted in a reflex response; there was always an increase in heart rate and usually an increase in arterial pressure. It was shown that the afferent pathway of the reflex was in the vagus nerves and the efferent pathway in the sympathetic nerves to the heart. It was thought that the receptors most likely to be involved in this reflex were the so-called left atrial receptors which have been shown to be clustered around the pulmonary vein–left atrial junctions in the dog (Coleridge *et al.* 1957). However, as was discussed fully in a previous paper (Ledsome & Linden, 1964*b*), it was not possible to assess the distending force exerted by small balloons inflated in the pulmonary veins.

In the investigation described in this paper an attempt was made to isolate at least some of the left atrial receptors in an isolated pouch of part of the left atrium and then to stimulate these receptors by raising the pressure within the pouch and stretching the atrial wall in a pulsatile manner. An increase in heart rate was observed in response to every dis-

tension. This response, though small, was in every way similar to that observed following distension of balloons in the pulmonary vein–atrial junctions (Ledsome & Linden, 1964*b*). The response was abolished or much reduced by section of the left vagus nerve at the level of the upper border of the aorta. The left vagus nerve at this level includes the recurrent laryngeal nerve, the thoracic vagus nerve and the ventrolateral cervical cardiac nerve (Mizeres, 1958); these nerves all contain afferent and efferent sympathetic fibres. However, it was shown (Ledsome & Linden, 1964*b*) that the response to pulmonary vein distension could also be abolished by cutting or cooling one or other vagus nerve in the neck confirming the fact that the afferent limb of the reflex was in the vagus nerves and that at least some of the fibres are to be found in the cervical vagus nerves. That these results were not due to interruption of efferent vagal pathways was shown by demonstrating a functioning vagal efferent pathway to the heart after the response to pulmonary vein distension had been abolished (Ledsome & Linden, 1964*b*). These results support the view that the afferent pathway of the reflex response to distension of the pouch of the left atrium is in the vagus nerves.

Bretylium tosylate, 10 mg/kg, in this type of preparation is known to block post-ganglionic sympathetic nerves without affecting afferent nerves or heart rate changes mediated through the vagus nerves (Ledsome & Linden, 1964*a*) or effects produced by the increased release of catecholamines from the adrenal medulla (Boura & Green, 1959). Propranolol in a dose of 0·5 mg/kg has been shown to reduce greatly the response of an increase in heart rate brought about by stimulation of the sympathetic nerves to the heart (Ledsome, Linden & Norman, 1965). Each of these drugs in these doses abolished the response of an increase in heart rate brought about by distension of the pouch of the left atrium suggesting that the efferent pathway of this reflex is in the sympathetic nerves to the heart.

The response in the present investigation was small; the average increase in heart rate was 10·3 beats/min compared with the reported response to balloon distension (Ledsome & Linden, 1964*b*) of a mean change of 24 beats/min (range, 2–89 beats/min). The differences in the increases in heart rate may be associated with the different control heart rates in the two groups: 137 beats/min (range 88–236) in the present series, 96 beats/min (range 36–168) in the previous series. However, several other factors may be involved. The number of receptors in the pouch was probably less than the number affected by the balloons. Also some receptors and/or some nerves may have been destroyed by the clamp applied to the atrium with the result that not all the receptors within the pouch may have been responding. In spite of the fact that the perfusing Ringer–Locke solution

was aerated with 5% CO_2 in O_2 and the fact that bright red blood oozed from the cut atrial muscle of the pouch at the end of the experiment it is not certain that *all* the receptors included in the wall of the pouch were viable. However, it is certain that some receptors were being stimulated by the pulsatile distension of the pouch because the increase in heart rate was shown to be mediated by a reflex.

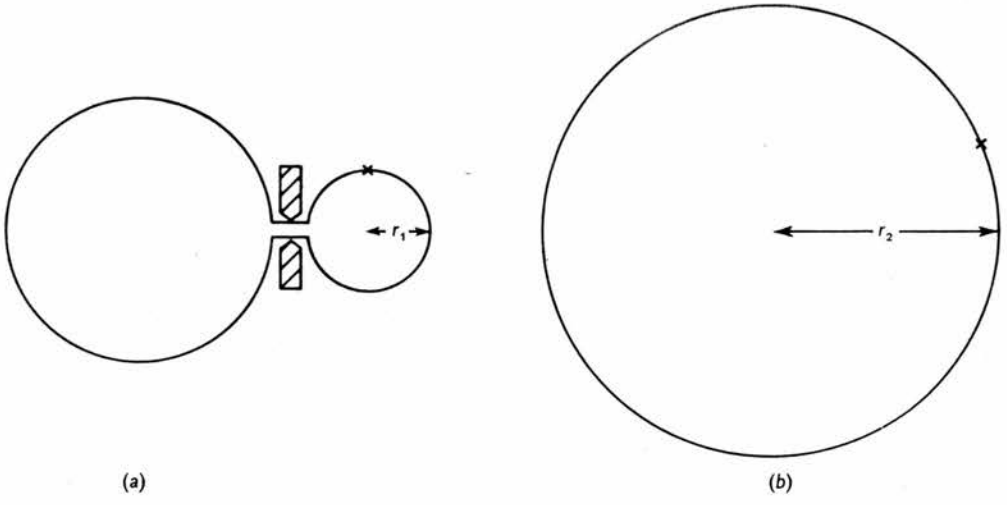

Fig. 3. Diagram representing the left atrium as a cylinder cut transversely. (*a*) With a clamp isolating a pouch of the left atrium; (*b*) with clamp removed. The ratio $r_1:r_2$ is 0·4:1·5. The tension in the atrial wall at the point × in each figure is proportional, at equivalent pressure, to the radius.

It may be argued that pressures in the left atrial pouch which were used to induce the reflex response were unphysiologically high and they are higher than the pressure usually observed in the left atrium of the anaesthetized dog (up to 20 cm H_2O). However it must be remembered that the pouch was small and the distended shape was almost cylindrical—similar to that of a pencil 2 cm long—with a radius of about 0·4 cm. If it is now assumed that the whole left atrium be of a similar cylindrical shape of larger size, say about 1·5 cm radius, a rough calculation according to the Law of Laplace (tension = radius × distending pressure) suggests that to maintain the same tension in the wall at any point (e.g. point *X* in Fig. 3) in the pouch (small chamber (*a*) in Fig. 3), as in the atrium (large chamber (*b*) in Fig. 3), would require 3–4 times more pressure within the pouch than within the atrium (given that r_1/r_2 was 0·4/1·5). This calculation is obviously an approximation but it indicates that the tension in the wall of the left atrial pouch (and therefore the degree of stretch) may be within the

normal range of tension, even though the pressure within the lumen is much higher.

It is concluded that the receptors most likely to be stimulated by distension of the left atrial pouch, and to be involved in the reflex increase in heart rate described, are those receptors situated at the junction of the left atrium and pulmonary veins—the left atrial receptors.

The authors are indebted to Mr G. Wade for technical assistance and are grateful for support from a Public Health Service Research Grant (no. H 6106) from the National Heart Institute, U.S.A., the British Heart Foundation, the Medical Research Council and the Wellcome Trust.

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J. Physiol. (1968), **198**, *pp.* 487–503
With 7 text-figures
Printed in Great Britain

THE ROLE OF LEFT ATRIAL RECEPTORS IN THE DIURETIC RESPONSE TO LEFT ATRIAL DISTENSION

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(*Received* 24 *April* 1968)

SUMMARY

1. The diuretic response to distension of the whole left atrium caused by obstruction of the mitral orifice has been compared with the effects of distension (by means of small balloons) of the left pulmonary vein/left atrial junctions.

2. Distension of the pulmonary vein/atrial junctions caused an increase in heart rate and a diuresis similar to but smaller than that caused by mitral obstruction.

3. Section of both ansae subclaviae prevented the increase in heart rate produced by distension of the pulmonary vein/left atrial junctions but had little effect on the diuretic response either to pulmonary vein distension or to mitral obstruction.

4. A diuretic response to mitral obstruction could be demonstrated after all nerves from the lungs had been cut but not after the vagus nerves had been cut at levels likely to interrupt the majority of afferent fibres from left atrial receptors.

5. The results support the view that stimulation of left atrial receptors is a major factor in the production of a diuretic response to mitral obstruction.

INTRODUCTION

Partial obstruction of the mitral orifice causes an increase in urine flow in anaesthetized (Henry, Gauer & Reeves, 1956) and unanaesthetized dogs (Lydtin & Hamilton, 1964), but the mechanisms by which the diuresis is produced have not been satisfactorily explained (Ledsome, Linden & O'Connor, 1961). It has been suggested (Henry & Pearce, 1956) that the afferent mechanism involves left atrial distension and stimulation of receptors in the left atrium. The majority of these receptors are situated in the sub-endocardial tissue at the junctions of the pulmonary veins and the left atrium (Coleridge, Hemingway, Holmes & Linden, 1957).

Recently it has been shown that when the pulmonary vein/left atrial junctions are distended by means of small balloons the atrial receptors are strongly stimulated (Kidd, Ledsome & Linden, 1966) and there is a reflex increase in heart rate (Ledsome & Linden, 1964, 1967). The afferent limb of this reflex is in the vagus nerves and the efferent limb is solely in the cardiac sympathetic nerves.

The present investigation was carried out to determine whether distension of the pulmonary vein/atrial junctions could cause diuresis and thus provide further information about the afferent mechanism by which left atrial distension causes diuresis.

METHODS

Dogs of 12–26 kg were given a subcutaneous injection of morphine sulphate (0·5 mg/kg). One hour later under local anaesthesia (decicain 2 %) a catheter was inserted through a saphenous vein into the inferior vena cava and each animal was anaesthetized by an intravenous infusion of a solution of chloralose (British Drug Houses): dose 10 ml. = 0·1 g/kg body wt. in sodium chloride solution (0·6 g/100 ml.). Subsequently during the experimental procedures a steady state of light anaesthesia and fluid input was maintained by the infusion every 10 min of 1·5 ml./kg body wt. of either sodium chloride solution (0·6 g/100 ml.) or the chloralose solution. As soon as possible after the induction of anaesthesia artificial respiration was started with a mixture of 40 % oxygen in air, humidified at room temperature and supplied from a Starling 'Ideal' pump, the rate (about 18/min) and stroke (about 50 ml./3 kg body wt.) of which were adjusted approximately to equal that of the animal's spontaneous respiration. When the chest was opened a resistance to expiration was provided by placing the expiratory outlet from the respiratory pump under 2–3 cm of water.

At intervals during the procedures samples of arterial blood were taken and pH, P_{CO_2}, P_{O_2} and total carbon dioxide content measured; the methods used have been described previously (Norman, Ledsome & Linden, 1965). Appropriate adjustments were made to the respiratory pump or small infusions (10–15 m-equiv) of sodium bicarbonate solution (1 M) were given to maintain P_{CO_2} between 35 and 40 mm Hg and pH within the range 7·3–7·4; no adjustments were made during the control and experimental periods.

The left side of the chest was opened in the fifth intercostal space and a small balloon placed in each of three pulmonary veins (Ledsome & Linden, 1964). In ten experiments a larger balloon was also inserted into the left atrium through the appendage (Ledsome *et al.* 1961).

Femoral arterial pressure was recorded through a metal cannula (Inconel; Johnson, Matthey & Co., London: 1·5 mm bore) and mean left atrial pressure through a 15 cm length of nylon tubing (Portex Plastics, No. 4 surgical nylon). To each cannula was attached a Statham Strain gauge (Model P 23 Gb) and after amplification by means of a carrier amplifier (S. E. Laboratories, Feltham, Middlesex) the pressure was recorded on an ultra-violet light recorder (S. E. Laboratories). The frequency response of the system recording femoral arterial pressure obtained by the method of Linden (1959) was flat (± 5 %) to better than 60 c/s. Mean pressure was obtained electrically by passing the amplifier output through an RC network.

In three experiments the right side of the chest was opened in the fourth intercostal space and a fine stainless-steel wire placed around the right vagus nerve immediately above the root of the right lung and around both roots of the right ansa subclavia. Similar wires were also placed around the left vagus nerve at the level of the upper border of the aorta and

around both roots of the left ansa subclavia. These nerves could then be cut without disturbing the animal by drawing the looped wire through a metal tube (1·5 mm bore) the end of which had been sharpened.

In two experiments the vagus nerves were cooled; the right vagus nerve in the neck and the left vagus nerve at the level of the upper border of the aorta were each placed on a silver-plated copper block which was maintained at 5° C by means of a thermo-electric cooling module (De La Rue Frigistor Ltd., Berks.)

In three dogs a soft polyethylene catheter was placed with the tip in the posterior part of the pericardial cavity overlying the pulmonary veins; the pericardium was then sutured and closed around the catheter. A solution of decicain 2 % was injected through this catheter at an appropriate time to block conduction in nerve fibres in this area.

During the surgical procedures, about 2 hr, the animals received a slow infusion of dextran ('Dextraven', Benger Laboratories Ltd., or 'Dextran 150 in 6 % dextrose', Fisons) of approximately 10 % of their estimated blood volume (100 ml. dextran for 13 kg body wt.). The electrocardiogram was recorded from leads on the forelegs and chest wall; records were taken every 10 min and heart rates were counted from the electrocardiogram over periods of at least 30 sec. Rectal temperature was maintained at 38° C (±1·5° C) by adjusting heating lamps above and beneath the animal.

Each ureter was catheterized through a flank incision and urine volume measured every 10 min. Urine was analysed for sodium, using a sodium electrode (Electronic Instruments Ltd., BH 104 glass). The electrode was calibrated with gravimetrically prepared sodium chloride solutions covering the range 10–200 mM. The results obtained by this method for urinary sodium are slightly lower than those obtained by the flame photometer (Moore & Wilson, 1963). However, changes in urinary sodium concentration are reliably indicated by the electrode.

RESULTS

Distension of the pulmonary vein/atrial junctions. The left pulmonary vein/left atrial junctions were distended by means of small balloons in ten dogs. Each balloon was distended with 1 ml. saline and the distension was maintained for 30 min; fourteen distensions were made in the ten dogs. In every experiment the heart rate increased within 1–2 min of distending the balloons and decreased over 1–5 min after the distension was ended. Although there were variations in the heart rate over the 90 min of each experimental period, this general pattern was always seen. The average of the heart rates over the 30 min before distension of the balloons was 117 beats/min, increased to an average of 157 beats/min over the 30 min for which the balloons were distended, and decreased to an average of 135 beats/min for the 30 min after deflation of the balloons. Thus the average change in heart rate was an increase of 21 beats/min. The magnitude of this increase in heart rate and the characteristics of the onset and decline of the heart rate changes were similar to those previously described for a series of shorter (3 min) distensions (Ledsome & Linden, 1964). There were no significant changes in mean arterial pressure during the distensions. During the fourteen tests there were only small changes in urine flow (Fig. 1) and in only one experiment was there an increase in urine flow during the experimental period to more than twice the flow during the

control periods. The average change in urine flow was from 0·24 ml./min in the control periods (30 min before balloon distension and the 30 min after the expected diuresis) to 0·29 ml./min during the expected diuresis (the second 20 min of balloon distension and the first 10 min after distension).

A further six balloon distensions were made in five dogs in which both ansae subclaviae had been cut (Fig. 1). Three of these dogs had been previously tested with the ansae subclaviae intact; in the other two dogs

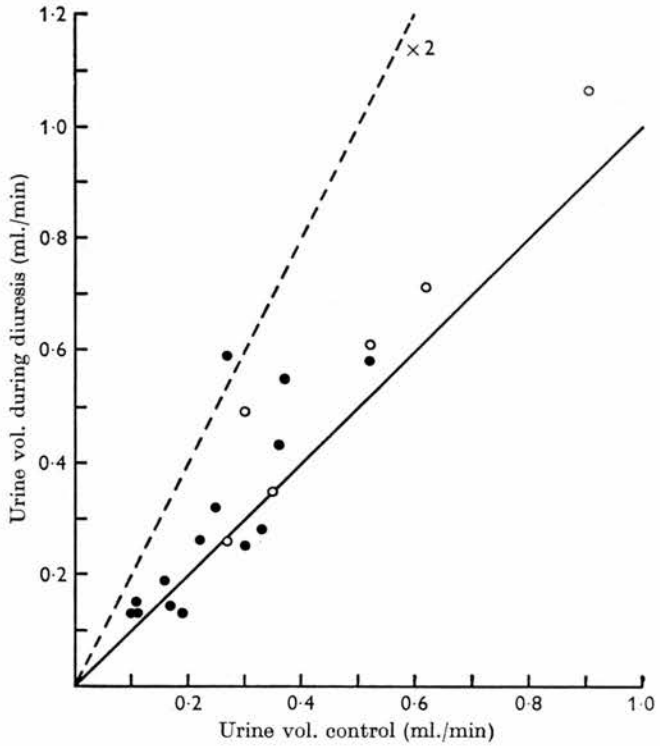

Fig. 1. Effects of distension of the pulmonary vein/atrial junctions. Urine volume during the period of the expected diuretic response compared with urine volume during the control periods (see text). Filled circles, fourteen tests in ten dogs; open circles, six tests in five dogs after cutting both ansae subclaviae. Continuous line is a line of no change. Increases of urine volume to twice the control value would fall on the dotted line.

the ansae subclaviae were cut at the beginning of the experiment. Cutting the ansae subclaviae prevented the increase in heart rate induced by pulmonary vein distension; the average heart rate before distension was 105 beats/min, 112 beats/min during distension, and 113 beats/min after distension. Thus the average change in heart rate was an increase of 3 beats/min. During each of the six tests there were only

small changes in urine flow but these were similar to those seen in the previous group (Fig. 1). The average change in urine flow was from 0·5 ml./min during the control periods to 0·59 ml./min during the experimental periods. On occasions when there were small increases in urine flow the urinary sodium concentration decreased so that sodium excretion remained relatively constant.

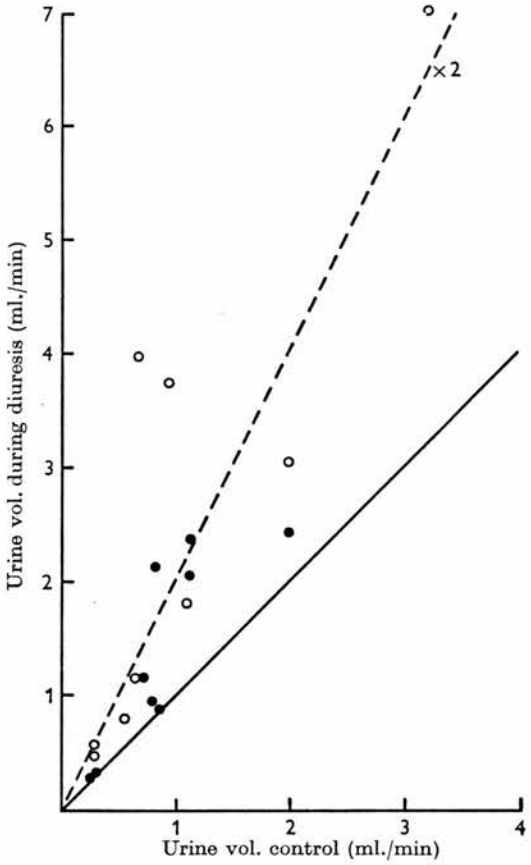

Fig. 2. Urine volume during the period of the expected diuretic response compared with urine volume during the control periods. Open circles, nine tests in five dogs of the effects of mitral obstruction; filled circles, nine tests in the same five dogs of the effect of distension of the pulmonary vein/atrial junctions.

Comparison of the effects of distension of the pulmonary vein/atrial junctions and mitral obstruction. The distension of the small balloons in the pulmonary vein/atrial junctions had resulted in only small changes in urine flow in most of the preparations. Distension of the large balloon which blocked the mitral orifice was known to cause greater responses (Ledsome *et al.* 1961). Because of the protean nature of this response and to eliminate the doubt

that the animals in which the pulmonary/vein atrial junctions had been distended were less 'responsive', the two methods of stimulation were compared in the same animals. Seven animals were prepared in which small balloons were inserted into the pulmonary vein/atrial junctions and in which a large balloon was placed in the left atrium. Tests were made alternately of the effects of distending the balloons in the pulmonary vein/atrial junctions for 30 min and of inflating the large balloon for 30 min. The large balloon was distended with saline (about 1 ml./kg body wt.) until it caused obstruction of the mitral orifice such that there was a rise in left atrial pressure of about 20 cm H_2O as described previously (Ledsome *et al.* 1961). A period of at least 90 min was allowed between the end of one test and the beginning of the next. Two experiments were abandoned, one when the dog died after the large balloon had burst, a second in which the dog had a very low urine flow, less than 0·1 ml./min, and showed no diuretic response to mitral obstruction.

The results from five dogs are therefore described. In two dogs mitral obstruction was tested first, in the other three dogs the pulmonary vein balloons were distended first. A total of nine tests of mitral obstruction and nine tests of pulmonary vein distension was made in the five dogs. The changes in urine flow which occurred are shown in Fig. 2. Distension of the small balloons in the pulmonary veins caused a small increase in urine flow in five of the nine tests, whereas mitral obstruction caused an increase in urine flow in all nine tests. The two groups of tests are further compared in Fig. 3, which shows the averaged values of the measured parameters during the control and experimental periods in all eighteen tests. Thus, whereas distension of the pulmonary veins caused an average increase in urine flow to 1·5 times the control flow, mitral obstruction in the same dogs caused an average increase to 2·7 times the control flow The time course of the changes was similar in the two groups of tests and there were only small changes in sodium excretion in both groups. The large increase in urine flow during mitral obstruction occurred in spite of a fall (5 mm Hg) in mean arterial pressure.

Because in these experiments there is often great variation in the responses of individual dogs and even in the same dog from time to time, quantitation of the response is always difficult and differences occurring in one dog are often more convincing than those for a group. The complete data for one experiment are therefore plotted in Fig. 4. In this experiment distension of the pulmonary vein/atrial junctions by means of small balloons caused an increase in heart rate and a small diuresis during each of two tests. However, mitral obstruction, although accompanied by a small (5 mm Hg) fall in mean arterial pressure, was associated with a very much larger diuretic response. Thus it may be concluded that distension

of the pulmonary vein/atrial junctions may produce a small diuresis with characteristics similar to those induced by mitral obstruction but that mitral obstruction provides a more effective stimulus.

Mitral obstruction and nerve section. In three dogs the effects of mitral obstruction for periods of 30 min caused by distension of a balloon in the left atrium were tested. Both ansae subclaviae were then cut and in the three dogs the test of mitral obstruction was repeated. Finally, the left

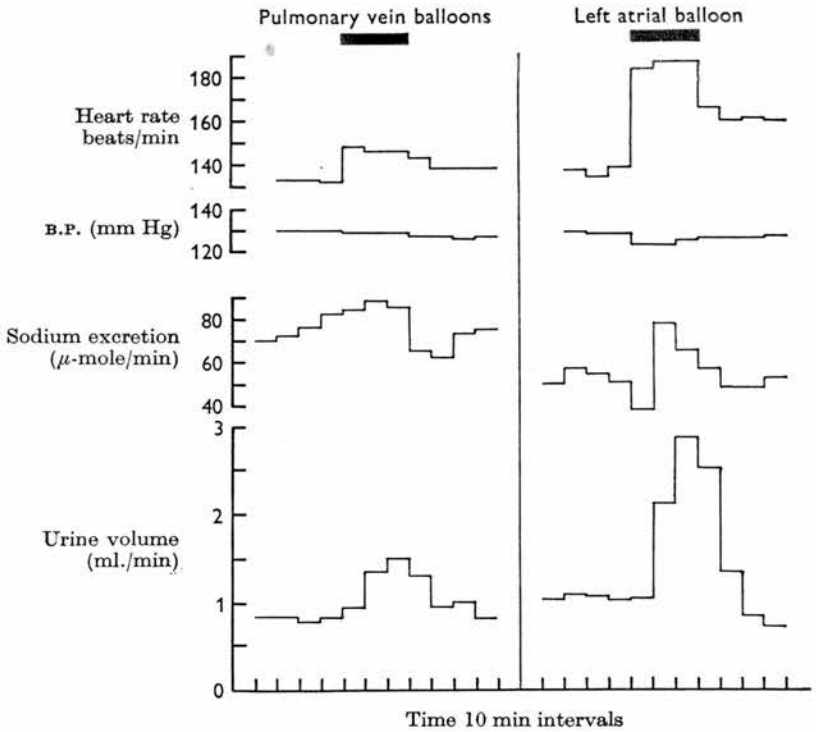

Fig. 3. Changes in heart rate, mean arterial pressure, sodium excretion and urine volume produced by distension of the pulmonary vein/atrial junctions and by mitral obstruction caused by inflating a balloon in the left atrium. Each horizontal line is the average of nine individual values from five dogs.

vagus nerve was cut at the level of the upper border of the aorta and the right vagus nerve was cut just above the lung root and the test of mitral obstruction repeated in all three dogs. One such experiment is shown in Fig. 5. Each period of mitral obstruction was accompanied by an increase in urine flow; at the same time the urine became more dilute. Although the absolute size of the diuresis was reduced in successive tests it should be noted that the urine flow during the control periods was also gradually falling so that the size of each diuresis relative to its control periods changed

considerably less. Also the first test in this dog produced the largest diuresis yet seen and the diuresis was followed by an increased sodium excretion lasting for about 1 hr. Two other dogs which also produced a large diuresis each showed similar increases in sodium excretion. This change may have

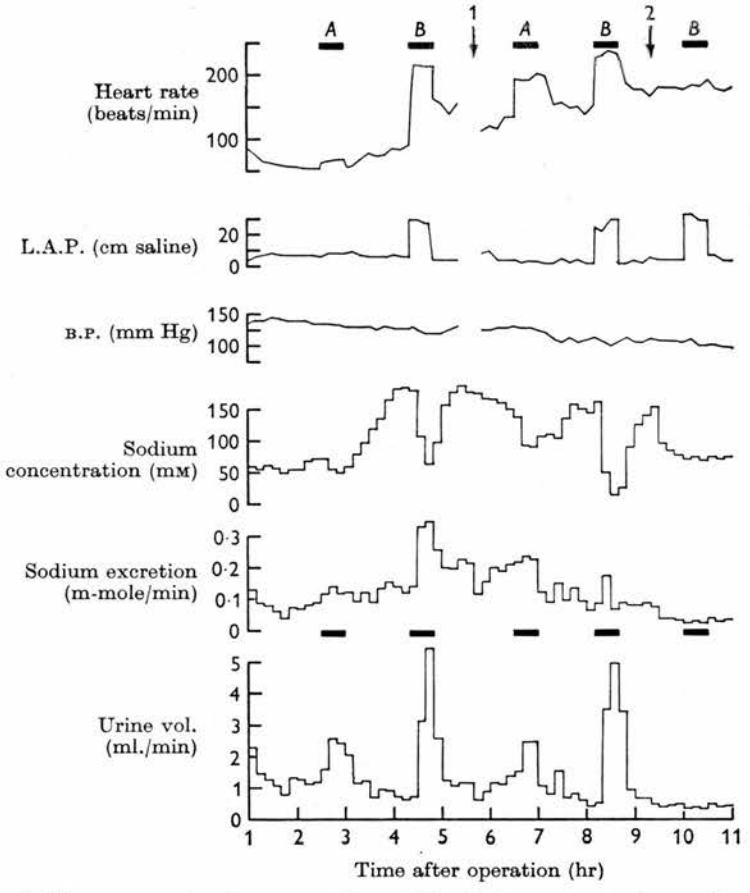

Fig. 4. Change occurring in measured variables during one experiment. During *A* small balloons were distended in the pulmonary vein/atrial junctions. During *B* a balloon was distended in the left atrium to cause mitral obstruction. There is a break in the record at '1', when the atrial balloon was replaced because it leaked. At '2' the right vagus nerve was cut in the neck and the left vagus nerve was cut at the level of the upper border of the aorta.

been associated with the large diureses produced in these animals, as it was not apparent in the previous investigation (Ledsome *et al.* 1961). The changes in urine flow in the three dogs are shown in Table 1 (Expts. 53, 55, 56). The average increase in urine flow caused by mitral obstruction with all nerves intact was 220%; with both ansae subclaviae cut the average

increase during the diuresis was 140% and with the vagus nerves cut as described urine flow increased by 65%. The increases in urine flow which occurred in these three dogs with nerves intact were exceptionally large, and it should be noted that the increases which occurred in these dogs with the ansae subclaviae cut were still larger than for the average of the eight dogs with intact nerves in Table 1. The increase in heart rate caused by mitral obstruction was reduced after cutting both ansae subclaviae (Fig. 5). Where the nerves were intact the average heart rate in the three dogs during the control periods was 105 beats/min and increased to 172 beats/min during mitral obstruction; after cutting the ansae subclaviae heart rate increased from 109 beats/min to 137 beats/min during mitral obstruction.

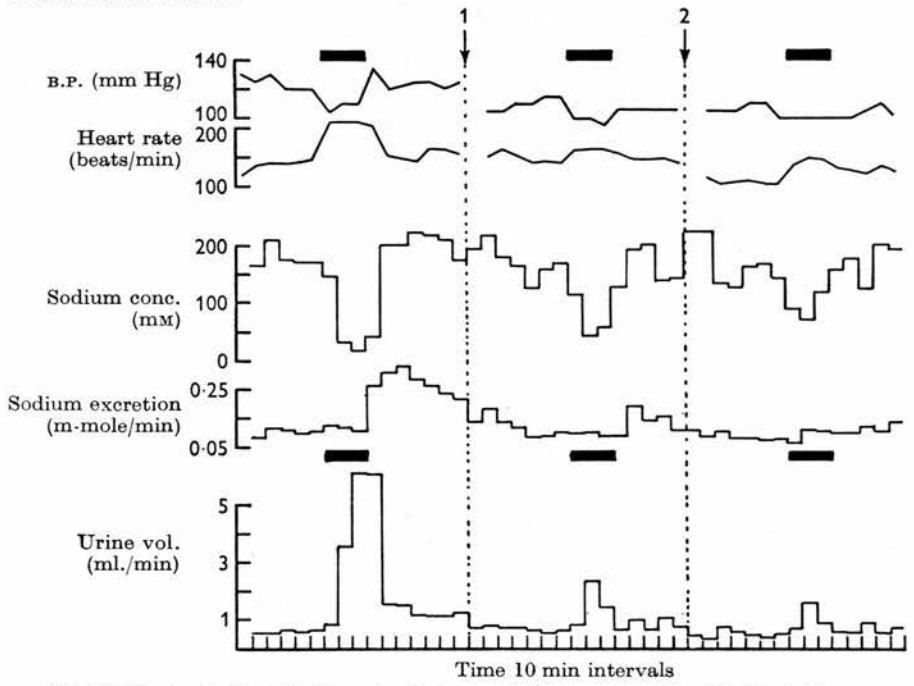

Fig. 5. Effects of left atrial distension before and after nerve section. Horizontal bars represent periods during which a balloon was inflated in the left atrium to cause mitral obstruction. At '1' both ansae subclaviae were cut; at '2' the right vagus nerve was cut immediately above the lung root and the left vagus nerve was cut at the level of the upper border of the aorta.

In four other dogs which had been shown to produce a diuretic response to mitral obstruction the right vagus nerve in the neck was cut or cooled to 6° C and the left vagus nerve at the upper border of the aorta was cut or cooled to 6° C. Mitral obstruction performed with the vagus nerves blocked at these sites was always accompanied by *a decrease* in urine flow

TABLE 1. Changes in urine volume during distension of a balloon in the left atrium before and after section or cold block of nerves. Numbers are average values, *A* for 30 min before balloon distension, *B* during last 20 min of balloon distension and the first 10 min after balloon distension, *C* the 30 min following *B*, % = percentage change in urine volume

	Urine volume (ml./min)															
									Vagi cut Left, at upper border of aorta							
	Nerves intact				Both ansae subclaviae cut				R. above lung root				R. in neck			
Expt. no.	*A*	*B*	*C*	%	*A*	*B*	*C*	%	*A*	*B*	*C*	%	*A*	*B*	*C*	%
53 (1)	1·89	3·40	1·95	+76	—	—	—	—	—	—	—	—	—	—	—	—
(2)	—	—	—	—	0·69	2·03	0·62	+210	—	—	—	—	—	—	—	—
(3)	—	—	—	—	—	—	—	—	0·38	0·83	0·50	+89	—	—	—	—
55 (1)	0·59	5·26	1·34	+440	—	—	—	—	—	—	—	—	—	—	—	—
(2)	—	—	—	—	0·62	1·52	0·86	+105	—	—	—	—	—	—	—	—
(3)	—	—	—	—	—	—	—	—	0·49	1·02	0·61	+85	—	—	—	—
56 (1)	1·46	2·89	0·86	+150	—	—	—	—	—	—	—	—	—	—	—	—
(2)	—	—	—	—	0·95	1·55	0·44	+110	—	—	—	—	—	—	—	—
(3)	—	—	—	—	—	—	—	—	0·41	0·46	0·44	+10	—	—	—	—
57 (1)	2·54	3·75	1·27	+97	—	—	—	—	—	—	—	—	—	—	—	—
(2)	—	—	—	—	—	—	—	—	0·43	0·77	0·40	+83	—	—	—	—
23 (1)	0·34	1·95	0·33	+480	—	—	—	—	—	—	—	—	—	—	—	—
(2)	—	—	—	—	—	—	—	—	—	—	—	—	0·48	0·38	0·45	−18
25 (1)	0·57	3·03	2·89	+75	—	—	—	—	—	—	—	—	—	—	—	—
(2)	—	—	—	—	—	—	—	—	—	—	—	—	0·54	0·13	0·38	−72
80 (1)	0·49	1·75	1·27	+100	—	—	—	—	—	—	—	—	—	—	—	—
(2)*																
(3)	0·47	1·18	0·45	+160	—	—	—	—	—	—	—	—	0·44	0·30	0·24	−12
81 (1)	1·08	2·95	2·19	+81	—	—	—	—	—	—	—	—	—	—	—	—
(2)*																
(3)	1·37	2·02	1·10	+64	—	—	—	—	—	—	—	—	1·39	0·88	2·05	−49
Average	1·08	2·81	1·36	+130	0·75	1·70	0·64	+141	0·43	0·77	0·49	+67	0·71	0·42	0·78	−43

* Vagi cooled to 6° C (thermode to 5° C).

(Figs. 4, 6). There was also a decrease in sodium excretion during mitral obstruction with the vagus nerves cooled. The diuretic response returned when the nerves were rewarmed (Fig. 6).

In three dogs the effect of mitral obstruction was tested before and after injecting 2 ml. decicain into the pericardial sac. In two dogs a diuretic response to mitral obstruction was obtained during the period before injection of decicain and about $2\frac{1}{2}$ hr later after the pericardial sac had

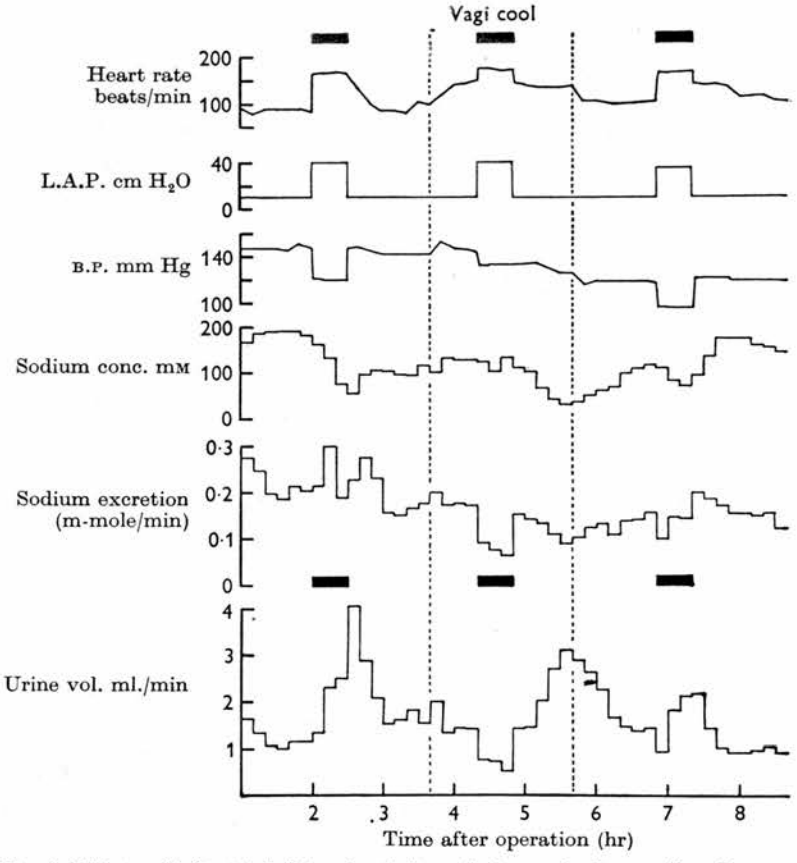

Fig. 6. Effects of left atrial distension before, during and after cooling the vagus nerves. A balloon was inflated in the left atrium to cause mitral obstruction during the periods indicated by the horizontal bars. Between the broken lines both vagus nerves were cooled to 6° C (thermode to 5° C): the right vagus nerve in the neck and the left vagus nerve at the level of the upper border of the aorta.

been washed out with saline and the effects of the decicain had worn off. Distension of the balloon in the left atrium during the experimental period 40 min after injection of the decicain caused a decrease in urine flow and sodium excretion in these two dogs. An example of one experiment is shown

(Fig. 7). In the third dog, although decicain abolished the response, the response did not return after washing out the pericardial sac. Examination at the end of the experiment showed the methylene blue which had been mixed with the decicain solution to be concentrated mainly around the intrapericardial portions of the pulmonary veins. However, there was

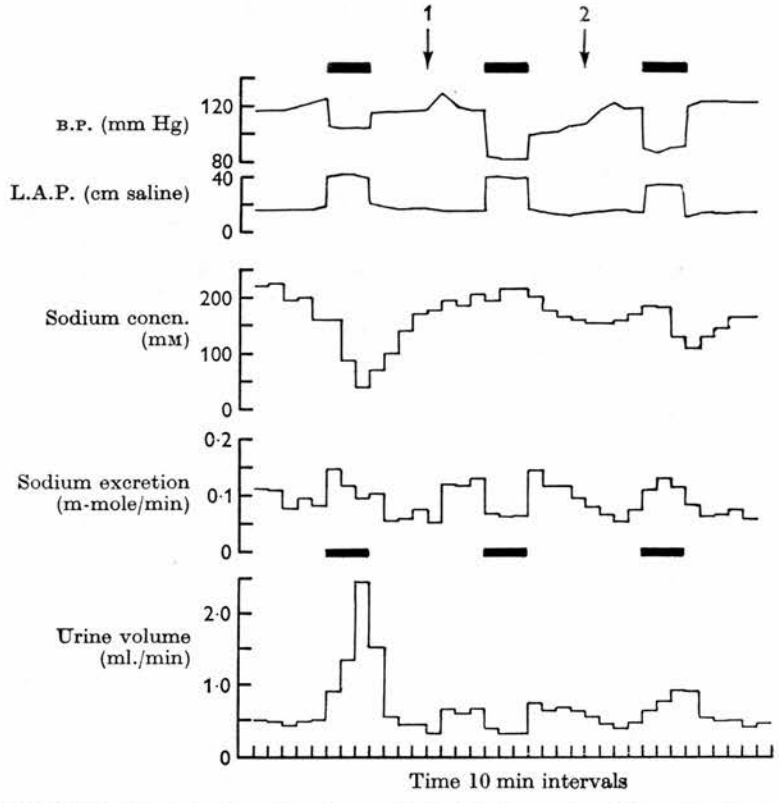

Fig. 7. Effect of an injection of local anaesthetic into the pericardial cavity. During the periods indicated by the bars the mitral orifice was obstructed by inflating a balloon in the left atrium. At the arrow marked '1', 2 ml. of a 2% (w/v) solution of decicain was injected into the pericardial cavity. At the arrow marked '2', the pericardial cavity was washed out with saline.

evidence that the decicain had not remained only within the pericardial sac, because the diaphragm was paralysed throughout the experimental period; presumably the phrenic nerve, which is closely applied externally to the thin pericardium just anterior to the lung roots and pulmonary veins, had been anaesthetized by the decicain. The diaphragm was contracting again during the last control period after the decicain had been washed out.

DISCUSSION

Inflation of a balloon in the left atrium partially to obstruct the mitral orifice causes an increase not only in left atrial pressure but also in the pressures throughout the pulmonary vascular bed (Henry *et al.* 1956). The existence of receptors stimulated by distension of the intrathoracic parts of the circulation is well established and in the dog they have been described in the pulmonary arteries (Coleridge & Kidd, 1960) and at the junctions of the venae cavae and pulmonary veins with the atria (Coleridge *et al.* 1957). Henry *et al.* (1956) and Henry & Pearce (1956) have suggested that the first stage in the production of diuresis by mitral obstruction is the stimulation of receptors in the walls of the left atrium. This localization of the afferent mechanism to the left atrium was achieved by comparing the effects of mitral obstruction with the effects of snaring the extrapericardial pulmonary veins or with the effects of blocking the pulmonary circulation with multiple emboli. The latter two procedures were ineffective in producing diuresis. However, these three manoeuvres are not strictly comparable, and it was therefore desirable to use a technique which as far as possible would limit the stimulus to receptors in the left atrium and would not obstruct blood flow through the left atrium. The technique of distending small balloons in the pulmonary vein/atrial junctions of the left side of the left atrium appeared to meet these requirements.

It is known that the distension of small balloons in the left pulmonary vein/atrial junction stimulates the so-called left atrial receptors vigorously to discharge (Kidd *et al.* 1966), and also causes a reflex increase in heart rate (Ledsome & Linden, 1964, 1967). The afferent limb of this reflex is in the vagus nerves and the efferent limb is solely in the sympathetic nerves to the heart. The changes in heart rate resulting from distension of the pulmonary vein/atrial junctions reported in this investigation entirely support these conclusions. The magnitude of the increase in heart rate and the characteristic onset and decline of the heart rate changes during the 30 min period of distension of the pulmonary vein/atrial junctions in the fifteen dogs were the same as those previously reported (Ledsome & Linden, 1964). Additional confirmation of the previous report was observed when, after cutting both ansae subclaviae in five dogs, the increase in heart rate produced by pulmonary vein distension did not occur. This technique of distension of the pulmonary vein/atrial junctions did not obstruct the flow of blood through the left atrium; in fact, concomitantly with responses of large increases in heart rate, it was observed that the pressure in the left atrium fell (Ledsome & Linden, 1964).

The results reported in this paper leave no doubt that distension of the pulmonary vein/atrial junctions can cause a diuresis with characteristics

similar, in all respects except its magnitude, to the diuretic response to mitral obstruction. However, the small size of the response to pulmonary vein distension and indeed the difficulty in demonstrating a response at all was surprising, particularly since the technique does not obstruct blood flow or cause a fall in arterial pressure. Arndt, Reineck & Gauer (1963) described a few experiments in which mitral obstruction had caused a severe reduction in cardiac output and antidiuresis, and it seemed likely that a fall in cardiac output could limit the diuretic response to mitral obstruction (Gauer & Henry, 1963). Several explanations for the small size of the diuretic response to pulmonary vein distension should be considered. First, pulmonary vein distension affects mainly those receptors on the left side of the left atrium whereas mitral obstruction will affect all the receptors in the atrium. Secondly, the small balloons may not have provided an adequate stimulus to all the receptors even on the left side of the left atrium. It has been shown that this technique can increase the discharge from single receptors from a resting discharge of about 4 impulses/beat to about 30 impulses/beat (Kidd *et al.* 1966); mitral obstruction has been shown to increase the discharge of single receptors from 8 impulses/beat to 20 impulses/beat (Henry & Pearce, 1956). There will be great variation in the responses of individual receptors but it is likely that pulmonary vein distension is at least as effective as mitral obstruction in causing a high rate of discharge from some of the receptors. Thus, it is probable that pulmonary vein distension produced less than half as much increase as mitral obstruction in the total number of afferent impulses discharged into the vagus nerves. Evidence that some receptors were stimulated in both these groups of experiments is given by the fact that there was always an increase in heart rate during every test and it is likely that this increase in heart rate resulted from the reflex described above. Thirdly, the animals and/or the kidneys of these animals may not have been in a suitable state to produce a diuresis. This criticism may be applied to the first group of dogs, in which the control urine flows were low (average 0·3 ml./min, see Figs. 1 and 2), but it cannot be applied to the group in which mitral obstruction was shown to be highly effective. Fourthly, the possibility remains that mitral obstruction achieves its effectiveness by stimulating not only atrial receptors but also by acting through some additional mechanism.

Because it has been shown that distension of the pulmonary vein/atrial junctions caused a reflex increase in heart rate with the efferent pathway in the cardiac sympathetic nerves (Ledsome & Linden, 1964) and that stimulation of cardiac sympathetic nerves has been shown to cause diuresis (Gilmore, 1959), the effects of cutting the ansae subclaviae on the diuretic responses to left atrial distension have been studied. The ansae

subclaviae in the dog contain most if not all of the sympathetic accelerator fibres to the heart (Mizeres, 1958). In the series of dogs in which only distension of the pulmonary vein/atrial junctions was tested, cutting both ansae subclaviae, though abolishing the heart rate response, did not appear to affect the already small diuresis. Quantitation of such small diuretic responses is difficult, but it may be significant that one of the largest responses occurred in a dog with both ansae cut (Fig. 1). In the dogs in which mitral obstruction was tested, cutting both ansae subclaviae did not prevent the appearance of large diuretic responses. Although the average change in urine flow after cutting the ansae subclaviae was rather less than in the tests with the nerves intact, this difference was due mainly to one control test in which there was an exceptionally large diuresis (Table 1). Thus cutting the ansae subclaviae did not appear to affect the *relative* size of the diuretic response to mitral obstruction although the increases in heart rate produced by the manoeuvre were reduced. The diuretic response to mitral obstruction is therefore not dependent upon an increased activity in efferent cardiac sympathetic nerves or upon afferent impulses travelling in the ansae subclaviae, but the increase in heart rate caused by mitral obstruction is partly dependent upon impulses travelling in the ansae subclaviae.

Cutting the thoracic vagus nerves above the lung roots cuts all afferent fibres from the lungs travelling in the vagus nerves. In addition, cutting the left vagus nerve at the level of the upper border of the aorta prevents most of the reflex increase in heart rate induced by pulmonary vein distension (Ledsome & Linden, 1964) and probably cuts most of the afferent fibres from the left side of the left atrium. The majority of receptors lying on the left side of the left atrium have their afferent fibres in the left vagus nerve (Coleridge, Coleridge & Kidd, 1964). In the present experiments cutting the right vagus nerve above the lung root and the left vagus nerve at the level of the upper border of the aorta may have reduced, but did not prevent, the diuretic response to mitral obstruction. The diuretic responses to mitral obstruction with these nerves cut also compared favourably with the responses to pulmonary vein distension although about half the fibres from left atrial receptors had been cut. When, in addition, the right vagus trunk was cut or cooled in the neck, there was no diuretic response to left atrial distension caused by mitral obstruction and indeed urine flow decreased. These results allow several conclusions to be reached. First, the diuretic response to mitral obstruction does not depend upon changes in activity in afferent fibres from the lungs. Secondly, sufficient afferent fibres to allow a diuretic response join the right vagus nerve between the lung root and the neck. Thirdly, cutting the left vagus nerve at the upper border of the aorta and not in the neck cuts the thoratic vagus

nerve, the recurrent laryngeal nerve and the inferior cervical cardiac nerve (Mizeres, 1955), but leaves intact most efferent vagal fibres and most afferent fibres from pulmonary arterial baroreceptors, aortic baroreceptors and aortic chemoreceptors (Coleridge *et al.* 1964). Reduction of the response after nerve section at this level is most readily explained in terms of interruption of afferent fibres from the left side of the left atrium (from the left atrial receptors), which have been shown by pulmonary vein distension to be capable of contributing to the diuretic response. Fourthly, impulses in afferent fibres from intrathoracic receptors which join the left vagus above the level of the upper border of the aorta may or may not contribute to the diuretic response but are not alone capable of initiating it. Lastly, the fact that an intrapericardial injection of a local anaesthetic prevented the diuretic response to mitral obstruction suggests that sensory nerve endings or fibres close to the heart are involved in the diuretic response. Because of the distribution of the dye used with the anaesthetic, the receptors and nerve fibres most likely to have been affected by the local anaesthetic are the left atrial receptors and/or their afferent fibres.

The experiments were designed to examine whether stimulation of left atrial receptors was involved in the diuretic response to left atrial distension. They do not provide any information on either the nature of the agent acting upon the kidney to produce the diuresis or whether or not the diuretic response may be considered as a specific reflex. The results support the view (Henry *et al.* 1956) that stimulation of left atrial receptors can cause diuresis and that such stimulation is a major factor in the production of the diuretic response to mitral obstruction. The possibility is raised that the peculiar effectiveness of mitral obstruction as a stimulus might depend in addition upon a change in the stimulus to other receptors. However, the experiments described provide no support for such a theory; indeed it is possible to speculate that stimulation of left atrial receptors is the only stimulus necessary to evoke the characteristic response.

The authors are indebted to Mr G. Wade for technical assistance and are grateful for support from a Public Health Service Research Grant (No. H6106) from the National Heart Institute, U.S.A., the British Heart Foundation, the Medical Research Council and the Wellcome Trust.

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J. Physiol. (1970), **207**, *pp.* 1–14
With 4 text-figures
Printed in Great Britain

THE EFFECTS OF DISTENSION OF THE PULMONARY VEIN–ATRIAL JUNCTIONS UPON PERIPHERAL VASCULAR RESISTANCE

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(*Received 14 April 1969*)

SUMMARY

1. Small balloons were inserted through the left pulmonary veins so as to lie at the pulmonary vein–left atrial junctions.

2. Distension of the balloons caused a reflex increase in heart rate. The afferent path was in the vagus nerves and the efferent path was in the cardiac sympathetic nerves.

3. Only small and variable changes in vascular resistance in a perfused hind limb accompanied the increase in heart rate when a steady state had been reached.

4. In about half of the experiments a transient vasodilatation was observed in the perfused hind limb, occurring immediately after distension of the pulmonary vein–atrial junctions and lasting about 22 sec.

5. The transient dilatation was due to a decrease in sympathetic vasoconstrictor nervous activity.

6. Stimulation of left atrial receptors causes an increase of sympathetic nervous activity to the heart but does not cause a corresponding increase in sympathetic nervous activity to the hind limbs.

INTRODUCTION

Stimulation of receptor areas in the heart and pulmonary circulation was generally thought to cause reflex bradycardia and systemic vasodilatation (Aviado & Schmidt, 1955; Heymans & Neil, 1958). More recently it has been shown that distending the left pulmonary vein–left atrial junctions by means of small balloons (Ledsome & Linden, 1964) and increasing the perfusion pressure in an isolated pouch of the left pulmonary

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veins and the adjacent part of the left atrium (Ledsome & Linden, 1967) caused a reflex increase in heart rate. The afferent path of this reflex was shown to be in the vagus nerves and the efferent path solely in the cardiac sympathetic nerves.

The present investigation was designed to determine if any changes in the vascular resistance of a perfused limb accompanied the reflex increase in the heart rate.

METHODS

Dogs of weight 13–21 kg were given a subcutaneous injection of morphine sulphate (0·5 mg/kg). About 1 hr later under local anaesthesia (decicain 2 %) a catheter was passed through a saphenous vein so that its tip lay in the inferior vena cava. The animals were anaesthetized by the infusion through this cannula of chloralose (0·1 g/kg; British Drug Houses). The chloralose was dissolved to make a solution, 1 g/100 ml., of sodium chloride solution (0·9 g/100 ml.). A state of light surgical anaesthesia was maintained during the experiment by further infusions of chloralose (about 10 mg/kg every 15 min). Following induction of anaesthesia the neck was opened in the mid line, the trachea cannulated and positive pressure ventilation started by means of a Starling 'Ideal' pump using air enriched to contain 40 % oxygen and humidified at room temperature. The rate of the pump was 18 strokes/min and the stroke volume was approximately 50 ml./3 kg body weight. When the pleura was opened a resistance to expiration was inserted equivalent to 3 cm water. In some of the dogs both common carotid arteries were dissected free for about 3 cm and a loose string was placed round each.

The chest was opened in the fifth left intercostal space and small balloons were inserted into each of three left pulmonary veins, as described by Ledsome & Linden (1964).

The left femoral artery in the groin was exposed for about 3 cm and any recurring branches were tied. The animal was then given an intravenous injection of Heparin (B.P. mucous; 500 i.u./kg followed by 50 i.u./kg every 30 min). A 3 mm bore stainless-steel cannula was tied into the proximal end of the left femoral artery and the blood thus received was pumped by a roller pump at a constant controlled flow through a 3 mm bore cannula tied in the distal end of the artery. This circuit was used in six dogs. In four other dogs the blood was received through a 3 mm bore nylon cannula which was passed up the left femoral artery so that its tip lay in the abdominal aorta. In these dogs the aorta was occluded just above its bifurcation either by tying the aorta on to the cannula or by injecting 2 ml. saline into a small balloon tied on the end of a length of 1 mm bore nylon tubing passed up the right femoral artery.

In a further five dogs the carotid arteries were perfused at various steady pressures. Blood was received through a cannula tied in the proximal end of the right common carotid artery. Some of this blood was pumped using a roller pump into a chamber at a rate which maintained a constant blood level in the chamber. The pressure of the air above the blood in this chamber was controlled. This provided a pressure-controlled perfusion system, the outlet of which led to a Y-shaped cannula with one limb in the cut distal end of each common carotid artery. Some of the blood obtained was used to perfuse the left hind limb at constant blood flow. The abdominal aorta was occluded at the bifurcation by a balloon.

Pressures in the cardiovascular system were recorded using Statham strain gauges (Model P 23 Gb) attached directly to the perfusion cannulae (femoral and carotid), to a 1·5 mm bore stainless-steel cannula tied in the right femoral or brachial artery

and to a nylon cannula (Portex No. 4, 6 in. long) inserted through the right femoral vein into the inferior vena cava. After amplification by a carrier amplifier (S.E. Laboratories, Feltham, Middlesex) the pressure signals were recorded on photographic paper by a direct-writing ultra-violet light-recorder (S.E. Laboratories). The frequency response of these systems as measured by the method of Linden (1959) was flat ($\pm 5\%$) to better than 60 c/s. Mean pressures were obtained by passing the output signal from the carrier amplifier through a simple R–C network with a time constant of 1 sec. Mean flow of blood to the limb was measured using a Medicon M-4000 electromagnetic flowmeter (Statham Instruments Inc.) with a cannulating transducer. Zero flow was recorded at intervals during the experiment and the flowmeter was calibrated using the animal's own blood at the end of the experiment. The output from the flowmeter and an e.c.g. obtained from chest-wall leads were also recorded. Heart rate was recorded using a Gilford Cardiotachometer triggered by the systemic arterial pressure pulse.

Arterial pH, P_{a,CO_2} and P_{a,O_2} were measured using the methods described by Norman, Ledsome & Linden (1965). pH was maintained between 7·30 and 7·40 by intravenous infusion of 1 M-$NaHCO_3$. P_{a,CO_2} was maintained between 36 and 40 mm Hg by adjusting the stroke of the respiration pump. P_{a,O_2} was always greater than 160 mm Hg. Oesophageal temperature was maintained at 38° C ± 1° C by adjusting heating lamps under the table.

RESULTS

After completion of the operative procedures, about 30 min were allowed for a steady state to be reached. Then the pulmonary vein–atrial junctions were distended for periods of 2·5 min by injecting 1 ml. saline into each balloon. Values of heart rate, limb perfusion pressure and systemic arterial pressure obtained after 2 min of distension of the pulmonary vein–atrial junctions were compared with the averages of the values obtained immediately before distension and 2 min after release of the distension. Mean values for the pressure readings were estimated over 30 sec and the heart rate was counted over the same period from the electrocardiogram. In five of the ten dogs there were transient changes in arterial pressure and limb perfusion pressure occurring immediately after the balloons were inflated. Results will therefore be presented of measurements made after a steady state had been reached (2 min) and a separate description will be given of changes occurring transiently following distension of the balloons.

The effects of distension of the pulmonary vein–arterial junctions for 2·5 min. Measurements were made of heart rate, arterial pressure and perfusion pressure in the limb in the control periods and during distension of the pulmonary vein balloons as described. The values of the change in heart rate and the percentage change in perfusion pressure to the limb in each test are compared in Fig. 1. This Figure shows the results of the first three balloon distensions made in each of ten dogs. This selection has been made to avoid overweighting the average values with results from those animals in which more tests were made. During distension of the pul-

monary vein–arterial junctions there was always an increase in heart rate compared with the control values. The average increase was 34·5 beats/min (S.E. of mean ±2·9, range 5–68) from a control heart rate of 127 beats/min (S.E. of mean ±6·6, range 61–193). Changes in the perfusion pressure to the limb (flow remained constant) were usually small and variable. The average perfusion pressure during distension of the pulmonary vein–atrial junctions was not significantly different from that during the control periods. However, when the values during distension

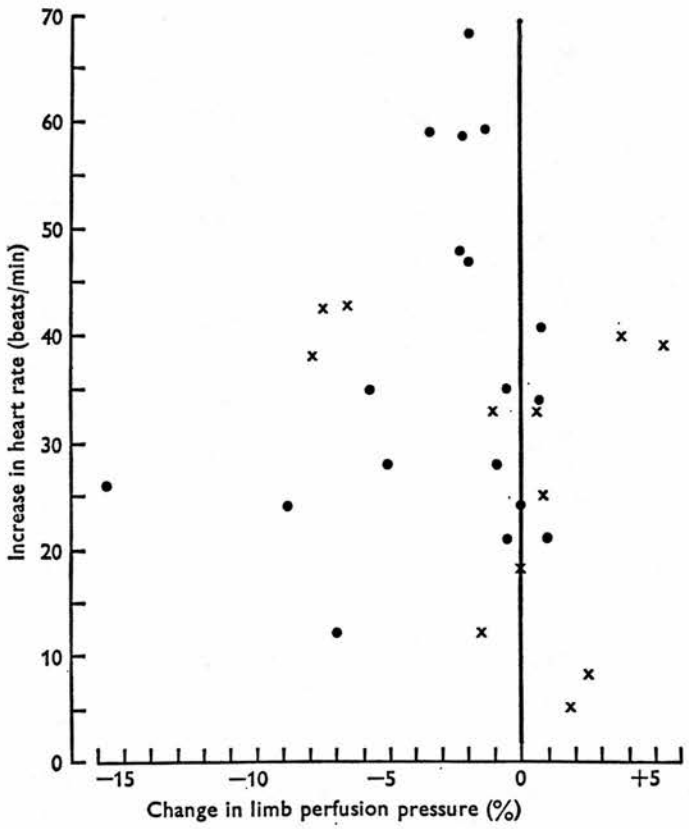

Fig. 1. Effects of distension of the pulmonary vein–atrial junctions; three distensions in each of ten dogs. 'Increase in heart rate' is the heart rate 2 min after distension minus the average of the heart rates immediately before distension and 2 min after release of distension. 'Change in limb perfusion pressure' is the % change in limb perfusion pressure after 2 min of distension of the pulmonary vein–atrial junctions from the average of the values of the perfusion pressure immediately before distension and 2 min after release of the distension. ● denotes observations made in experiments in which the abdominal aorta was not occluded and ×, those in which the aorta was occluded at the bifurcation.

of the pulmonary vein–atrial junctions were expressed as a percentage of the control values (as in Fig. 1), the paired differences between the control and experimental values in each test showed a small but statistically significant difference ($P < 0{\cdot}01$). The average difference was a decrease in perfusion pressure of 2·21 % (S.E.M. of mean ± 0·78, range 15·7 % decrease–5·3 % increase); that is, there was a small decrease in vascular resistance in the limb during distension of the pulmonary vein–atrial junctions. There were no significant changes in mean systemic arterial pressure. The average change was a decrease of 0·8 % (S.E. of mean ± 0·44, range 8·7 % decrease–3·8 % increase). There was an average decrease in systolic pressure of 8·5 mm Hg (S.E. of mean ± 1·4, range 20 mm Hg decrease to 6 mm Hg increase). Pulse pressure decreased by an average of 12·2 mm Hg (S.E. of mean ± 1·5 range 0–32 mm Hg); i.e. diastolic pressure increased.

An example is given in Fig. 2 of parts of the record obtained in one experiment in which a large increase in heart rate occurred on balloon distension. Although the heart rate increased by 68 beats/min, there was only a small decrease (3 mm Hg) in femoral perfusion pressure. Mean systemic pressure remained constant, but there was a decrease in systolic and pulse pressure with an increase in diastolic pressure.

The responses obtained in experiments in which the abdominal aorta was occluded were similar to those obtained with the aorta not occluded (Fig. 1). In experiments in which the aorta was occluded, the pressure in the femoral perfusion cannula fell to the level of the venous pressure when the femoral perfusion pump was stopped, thus demonstrating the absence of significant anastomoses between the systemic arteries and the perfused limb.

Immediate effects of distension of the pulmonary vein–atrial junctions. In five out of the ten dogs there was a transient decrease in limb perfusion pressure and mean systemic pressure immediately following distension of the pulmonary vein–atrial junctions (Fig. 3). In those dogs in which a transient response was observed, it occurred following each test of balloon distension. The average maximum fall in perfusion pressure was 30 mm Hg (range 12–50 mm Hg); this returned to a steady-state value after 22 sec (range 15–50 sec). The changes in systemic pressure were of a similar magnitude and duration. There was no transient heart rate change; this increased and remained faster for as long as the balloons were distended. In about half the cases in which a transient decrease in perfusion pressure occurred, this response followed an abnormally large pulse beat or a series of extrasystoles initiated by the distension of the balloons. However, such extrasystoles followed by heart beats with large pulse pressures occurred in about half the experiments without associated transient responses.

The nature of the reflex mechanism. In five of the dogs the right vagus

was cut in the neck and the left vagus nerve cooled to 5° C (Thermode to 4° C) for 5 min. This abolished all responses to distension of the pulmonary vein–atrial junctions, including the transient responses in the three dogs tested in which these were obtained. After the left vagus had been rewarmed, the increase in heart rate and the transient falls in systemic pressure and limb perfusion pressure were again observed on distension of the pulmonary vein–atrial junctions.

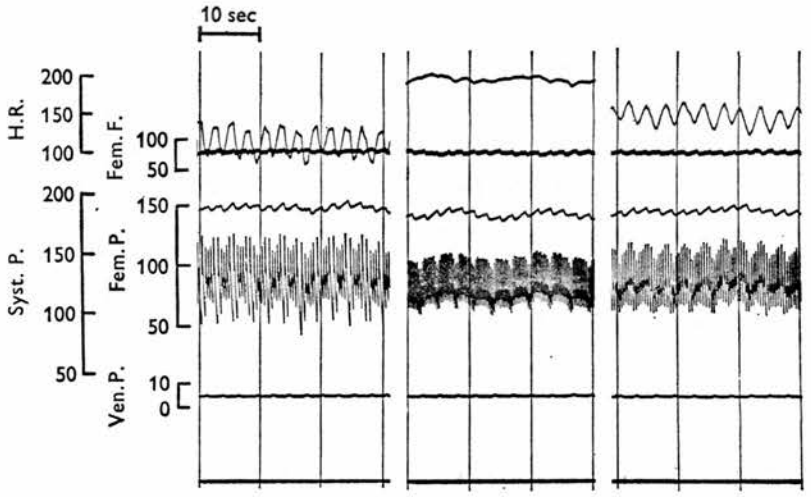

Fig. 2. Effects of distension of the pulmonary vein–atrial junctions in a dog in which a large increase in heart rate occurred. Dog 13 kg. Femoral artery perfusion, aorta not occluded. From above downwards heart rate, femoral perfusion flow, femoral perfusion pressure, systemic arterial pressure (recorded in the right femoral artery) and venous pressure. H.R., heart rate (beats/min); Fem. F., femoral perfusion flow (ml./min); Fem. P., femoral perfusion pressure (mm Hg); Syst. P., systemic arterial pressure (mm Hg); Ven. P., venous pressure (cm H_2O). First column recorded immediately before distension of the pulmonary vein–atrial junctions; second column, after 2 min of distension; third column, 2 min after release of distension.

In two dogs, propranolol (0·5 mg/kg) was given intravenously. In one dog the heart rate increase on distension of the pulmonary vein–atrial junctions was reduced from 38 beats/min immediately before propranolol to 12 beats/min after propranolol. In the other dog the heart rate increase was reduced from 81 beats/min to 16 beats/min. In neither of these experiments was a significant steady-state change observed in the perfusion pressure following balloon distension before or after injection of propranolol. The transient responses of a fall in systemic pressure and a decrease in perfusion pressure in the limb were unaffected by propranolol (two dogs) or an intravenous injection of atropine sulphate, 0·4 mg/kg (two dogs).

They were, however, abolished by an intravenous injection of bretylium tosylate, 10 mg/kg (two dogs).

In six of the dogs both common carotid arteries were clipped simultaneously. This always produced an increase in both systemic pressure and limb perfusion pressure. The average maximum rise in mean systemic pressure was 54 % (range 28–82 %) and the average maximum rise in limb perfusion pressure was 59 % (range 26–105 %).

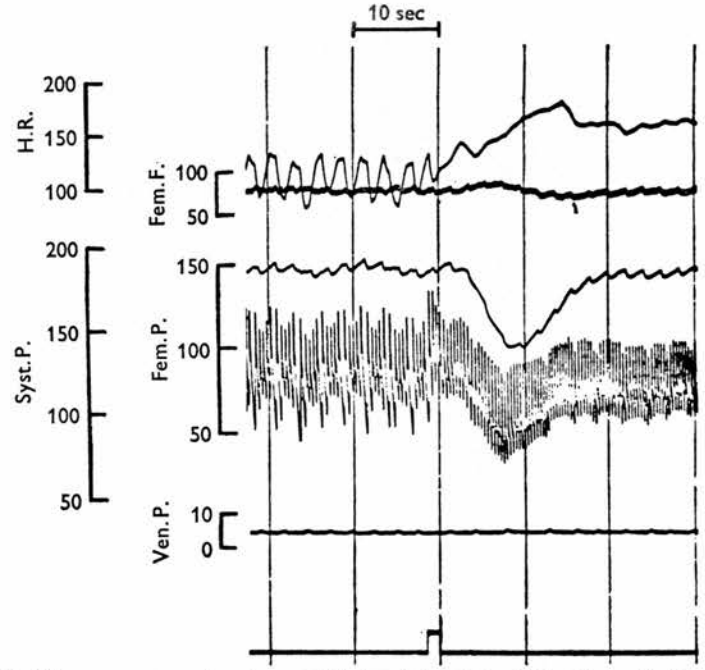

Fig. 3. Changes occurring immediately following distension of the pulmonary vein–atrial junctions. Record from same animal as in Fig. 2. Conventions as in Fig. 2.

Distension of the pulmonary vein–atrial junctions with controlled carotid perfusion. The effects were studied of distension of the pulmonary vein–atrial junctions in five dogs in which both common carotid arteries were perfused at controlled non-pulsatile pressures. The carotid perfusion pressure was set at various levels between 64 and 212 mm Hg. Increasing the carotid perfusion pressure always reduced the femoral perfusion pressure and the systemic arterial pressure. The effect on heart rate was less consistent. A large rise in carotid perfusion pressure always resulted in a transient bradycardia, but often there was little change in heart rate in the steady state. Distension of the pulmonary vein–atrial junctions at all carotid perfusion pressures always caused an increase in heart rate; the

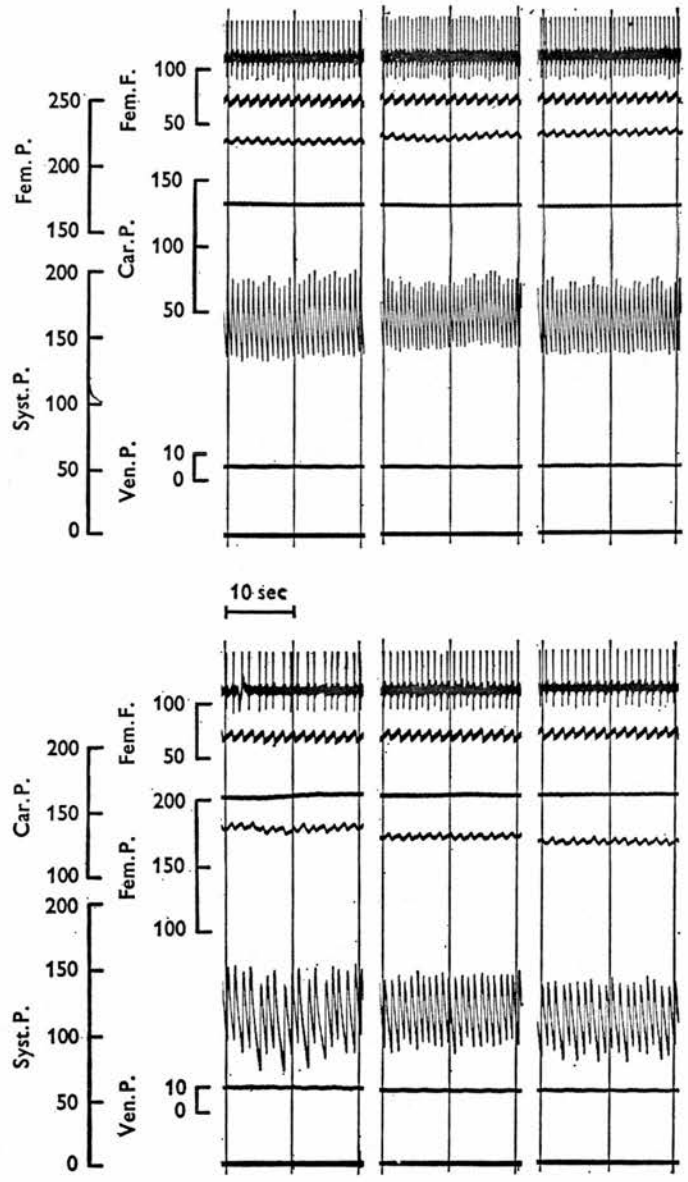

Fig. 4. Effects of distension of the pulmonary vein–atrial junctions at two different constant carotid perfusion pressures. Dog 16 kg. Femoral artery perfusion; aorta occluded at bifurcation. Upper records from above downwards, e.c.g., femoral perfusion flow, femoral perfusion pressure, carotid perfusion pressure, systemic arterial pressure (recorded in right brachial artery), and venous pressure. Lower records, e.c.g., femoral flow, carotid perfusion pressure, femoral perfusion pressure, systemic arterial pressure and venous pressure. Car. P., carotid perfusion pressure (mm Hg). Other conventions as in Fig. 2.

effects on limb perfusion pressure and systemic arterial pressure were always small and variable. Figure 4 shows a record obtained in one experiment in which an increase in heart rate was obtained on distension of the pulmonary vein–atrial junctions at two different carotid pressures. In this experiment, increasing the carotid pressure did reduce the heart rate as well as the limb perfusion pressure and the systemic arterial pressure. Distension of the pulmonary vein–atrial junctions resulted in an increase in heart rate but no definite effect on limb perfusion pressure or systemic arterial pressure at any carotid pressure.

TABLE 1. Effects of distension of pulmonary vein–atrial junctions at controlled carotid perfusion pressures. Results from five dogs in which carotid perfusion pressures were 132 mm Hg and below (sixteen tests) and 138 mm Hg and above (twenty-two tests). Average values given with ranges of individual observations. Control values are the averages of measurements made immediately before, and 2 min after, release of distension. Changes refer to the differences from the control values of measurements made after 2 min of distension

	Low carotid pressure	High carotid pressure
Carotid perfusion pressure (mm Hg)	85 (47–132)	168 (138–212)
Control heart rate (beats/min)	143 (82–207)	132 (54–202)
Change in heart rate (beats/min)	+24 (+9–+42)	+18 (+6–+35)
Control limb perfusion pressure (mm Hg)*	197 (145–232)	137 (90–184)
Percent change in limb perfusion pressure	−0·3 (−7·0–+3·4)	+3·2 (−3·5–+16·2)
Control mean systemic pressure (mm Hg)*	186 (161–202)	122 (82–180)
Percent change in mean systemic pressure	−4·1 (−9·3–+2·0)	−1·3 (−10·7–+9·0)

* Indicates that paired observations in each dog of values obtained at high and low carotid pressures are significantly different ($P < 0{\cdot}01$).

For analysis of the results, the experiments have been divided into those at carotid pressures up to 132 mm Hg (sixteen tests) and those at carotid pressures of 138 mm Hg and over (twenty-two tests). No significant differences were observed between the responses of heart rate, limb perfusion pressure or systemic arterial pressure to distension of the pulmonary vein–atrial junctions at high and low carotid pressures. It should be noted that in these five dogs there was no significant decrease in limb perfusion pressure during distension of the pulmonary vein–atrial junctions at either high or low carotid pressure. In fact, in the experiments carried out at high carotid pressure, the average change on pulmonary vein distension was a small increase in limb perfusion pressure (Table 1).

DISCUSSION

The balloons inserted through the left pulmonary veins were tied and clamped in such a position that distension by 1 ml. saline would distend the pulmonary vein–atrial junctions. The subendocardium at the pulmonary vein–atrial junctions was shown by Coleridge, Hemingway, Holmes & Linden (1957), using combined electrophysiological and histological techniques, to be the site where left atrial receptors were concentrated. Kidd, Ledsome & Linden (1966) showed that these left atrial receptors were strongly stimulated on distension by small balloons and that such stimulation was maintained as long as the distension continued. In the present experiments, the lung roots were tied tightly distal to the insertions of the balloons; it is unlikely that distension of these balloons would interfere with blood flow through the left atrium. It was considered likely that the responses obtained on distension of the balloons would be a result of stimulation of left atrial receptors.

Distension of the pulmonary vein–atrial junctions consistently caused an increase in heart rate. An increase in heart rate was not obtained when the pulmonary vein–atrial junctions were distended following division of the right vagus nerve and cold block of the left vagus nerve. The response was greatly reduced following injection of propranolol (0·5 mg/kg). Propranolol in this dose had been shown to greatly reduce, but not completely prevent, the increase in heart rate caused by stimulation of the cardiac sympathetic nerves (Ledsome, Linden & Norman, 1965). These results, therefore, confirmed the findings of Ledsome & Linden (1964) that distension of the pulmonary vein–atrial junctions caused an increase in heart rate by a reflex with an afferent path in the vagus and an efferent path in the cardiac sympathetic nerves.

In the steady state (after 2 min) the changes in vascular resistance in a perfused hind limb which accompanied the reflex increase in heart rate were variable (Fig. 1). However, there was a small but statistically significant decrease in vascular resistance in the limb. The change in vascular resistance could have been due to stimulation of atrial receptors as a result of the distension of the pulmonary vein–atrial junctions. Equally, the change may have been produced secondary to an increase in the stimulus to the arterial baroreceptors as a result of the increase in heart rate in spite of the decrease in arterial pulse pressure which occurred. It is well known that a decrease in pulse pressure leads to a decreased stimulus to the arterial baroreceptors (Ead, Green & Neil, 1952) but it is impossible to predict in any individual experiment whether the combination of a decrease in pulse pressure accompanied by an increase in heart rate would lead to a greater or lesser stimulation of the arterial baroreceptors. In

those experiments in which carotid arterial pressure was controlled, thus maintaining a constant stimulus to at least some of the arterial baroreceptors, no significant decrease in perfusion pressure in the limb was observed. Also in some experiments (Fig. 1, Table 1) there was an increase rather than a decrease in perfusion pressure in the limb; this was seen most frequently when the carotid perfusion pressure was controlled at high pressure. It cannot therefore be concluded that the small decrease in perfusion pressure observed in the steady state in the first group of experiments represents the direct reflex response to stimulation of left atrial receptors.

The small size of the changes in vascular resistance in the steady state could not have been due to damage to vasomotor nerves to the limb because large changes in vascular resistance occurred immediately following changing the pressure in the carotid arteries either by changing the carotid perfusion pressure or by occluding the common carotid arteries. In the experiments in which the carotid arteries were perfused at different pressures the limb vascular resistance was varied over a wide range. The absence of a reproducible vasomotor response on distension of the pulmonary vein–atrial junctions could not then be explained by the limb vessels being already in a state of maximal dilatation or maximal constriction.

The experiments also emphasize the difference between the relative effects upon the heart and peripheral vessels of distending the pulmonary vein–atrial junctions and of changing the carotid perfusion pressure. Distension of the pulmonary vein–atrial junctions consistently caused an increase in heart rate accompanied by only small and variable changes in vascular resistance; increasing the carotid perfusion pressure always caused a large decrease in vascular resistance and a decrease in heart rate to a usually smaller and more variable extent. These reflex effects of increasing carotid perfusion pressure are similar to the reflex changes induced when pressure is raised in an isolated carotid sinus preparation but it should be noted that in the present experiments pressure changes were induced throughout the cerebral vascular bed.

In about half of the experiments a transient vasodilatation was observed occurring immediately following distension of the pulmonary vein–atrial junctions. The transient vasodilatation was seen as a decrease both in limb perfusion pressure and in systemic arterial blood pressure of approximately equal magnitudes. This response persisted for an average of 22 sec. The occurrence and magnitude of a transient response were not related to the magnitude of the steady-state response of either vascular resistance or heart rate. However, in those experiments in which a transient vasodilatation was observed, it was noted that it occurred each time the pulmonary

vein–atrial junctions were distended. In these experiments, as the blood pressure and limb perfusion pressure fell, the heart rate increased. The heart rate, however, remained faster for as long as the stimulus was applied. The transient vasodilatation was still observed on distension of the pulmonary vein–atrial junctions following the intravenous injection of propranolol (0·5 mg/kg), which greatly attenuated the heart rate response. It was not modified by the intravenous injection of atropine sulphate (0·4 mg/kg) or by both propranolol and atropine. The response was no longer obtained following cutting or cooling both vagus nerves in the neck. It was also prevented by the intravenous injection of bretylium tosylate (10 mg/kg), which is known to block the efferent sympathetic vasoconstrictor nerves (Boura & Green, 1959). The transient vasodilatation following distension of the pulmonary vein–atrial junctions must, therefore, be dependent upon afferent impulses travelling in the vagus nerves and be effected by the release of sympathetic vasoconstriction.

The mechanism responsible for the transient vasodilatation remains in doubt. If the dilatation were due to stimulation of left atrial receptors then the transient nature of the response must be due to mechanisms other than adaptation of the receptors to the stimulus. One such mechanism could be a restoration of systemic pressure by the reflex action of the arterial baroreceptors. If this were the case it would be expected that the magnitude and duration of the vasodilatation would have been greater when carotid arterial pressure was maintained constant. It is possible that distension of the balloons caused temporary stimulation of receptors other than those at the pulmonary vein–atrial junctions. If there was excessive traction on the balloon catheters distension of the balloons could cause a small movement of the whole heart. Also injection of 3 ml. fluid into the balloons effectively increases the volume of the atrial contents and could lead to a temporary increase in ventricular volume, so that immediately at the time of distension the stimulus may not have been limited to the pulmonary vein–atrial junctions. Extrasystoles and an abnormally large pulse beat were sometimes observed at the time of balloon distension but the incidence of such changes was no higher in those tests in which a transient vasodilatation occurred than in those tests in which it did not occur. Sleight & Widdicombe (1965*a*, *b*) have described receptors in the epicardium and pericardium the discharge of which is stimulated by distension of the ventricle. It is possible that such epicardial or pericardial receptors could be stimulated on balloon distension; stimulation of epicardial receptors is said to cause bradycardia and hypotension; there is no knowledge of the reflex effects of stimulating pericardial receptors.

The mechanism responsible for causing a transient vasodilatation in some of the experiments thus remains a matter for speculation; it is

believed that it may not be due to stimulation of left atrial receptors. It may be relevant that in experiments performed by earlier investigators (Daly, Ludány, Todd & Verney, 1937; Aviado, Li, Kalow, Schmidt, Turnbull, Peskin, Hess & Weiss, 1951) in which hypotension was obtained on increasing left atrial and pulmonary venous pressures, the stimuli were applied for a short duration (20 sec) and responses of short duration resulted. Although in many cases the responses were capable of alternative explanations, it is possible that in some cases the hypotension reported may have been caused by stimulation of receptors similar to those responsible for causing the transient vasodilatation reported in the present experiments, i.e. they may not have been the result of stimulation of left atrial receptors. In these earlier experiments a slowing of the heart rate was obtained associated with the hypotensive response. It must be emphasized that, in the present experiments, even though a transient hypotension occurred in half the experiments, there was never even a transient bradycardia; the heart rate always increased following distension of the pulmonary vein–atrial junctions.

Distension of the pulmonary vein–atrial junctions has been shown to cause a reflex increase in heart rate as a result of an increase in sympathetic nervous activity to the heart. No evidence has been found of concomitant changes in the activity of sympathetic vasomotor nerves to the hind limb. There was no convincing evidence that the transient vasodilatation sometimes observed was caused by the same mechanism which is thought responsible for the increase in heart rate, that is, stimulation of left atrial receptors. The results are significant in that they demonstrate that the effects of stimulation of atrial receptors are unlike those of stimulation of other cardiovascular stretch receptors. This conclusion contradicts an opinion formerly held (Aviado & Schmidt, 1955; Heymans & Neil, 1958) that stimulation of all cardiovascular stretch receptors would be likely to result in similar responses. Also, this is believed to be the first example reported of a reflex response which causes an increase in activity in the cardiac sympathetic nerves and not in the vasomotor nerves to the limbs.

The authors are grateful to Miss H. Newton and Mr R. Hirst for technical assistance.

This work was supported by grants from the British Heart Foundation, The Medical Research Council, The Wellcome Trust and U.S.P.H.S. Grant No. TW 00234.

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THE EFFECTS UPON RESPIRATION OF DISTENSION OF THE PULMONARY VEIN-ATRIAL JUNCTIONS

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Abstract. Left atrial receptors in anaesthetized dogs were stimulated by distension of small balloons at the pulmonary vein-atrial junctions. No significant changes in respiratory rate or tidal volume were recorded although there was consistently a reflex increase in heart rate. It is considered unlikely that stimulation of left atrial receptors makes a significant contribution to the hyperpnoea of muscular exercise.

Cardiac reflex — Muscular exercise
Control of breathing — Pulmonary vein–atrial receptors

It has been reported that distension of the pulmonary veins and the left atrium may cause tachypnoea (DALY *et al.*, 1937). This view was supported by AVIADO *et al.* (1951) who suggested that stimulation of pulmonary venous receptors formed the afferent mechanism for reflex tachypnoea.

Recently it has been shown that distension of the left pulmonary vein-left atrial junctions in anaesthetized dogs causes a reflex increase in heart rate; the afferent path of this reflex is in the vagus nerves and the efferent path is in the cardiac sympathetic nerves (LEDSOME and LINDEN, 1964, 1967). The receptors most likely to be involved in this reflex are the left atrial receptors which are situated in the subendocardial tissue close to the junctions of the pulmonary veins with the left atrium (COLERIDGE *et al.*, 1957; KIDD, LEDSOME and LINDEN, 1966). The present investigation was designed to test whether the reflex increase in heart rate caused by distension of the pulmonary vein-atrial junctions was accompanied by changes in respiratory rate or tidal volume.

Methods

Dogs of 17–23 kg (average 19.6 kg) were given a subcutaneous injection of morphine sulphate (0.5 mg/kg). One hour later under local anaesthesia (decicain 2%) a catheter was inserted through a saphenous vein into the inferior vena cava and each animal

Accepted for publication 24 October 1969.

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was anaesthetized by an intravenous infusion of a 1% (1 g/100 ml) solution of chloralose (British Drug Houses; dose = 0.1 g/kg) in sodium chloride solution (0.9 g/100 ml). Subsequently a steady state of light anaesthesia was maintained by the infusion of chloralose (about 1 ml/kg of a solution of 1 g/100 ml) every 15 min. A tracheal cannula was inserted and during the operative procedures positive pressure-ventilation with air humidified at room temperature and enriched to contain 40% oxygen was maintained by a Starling "Ideal" pump. The rate of the pump was 18 strokes/min and the stroke volume (about 50 ml/3 kg body wt) was adjusted to keep arterial carbon dioxide tension at about 40 mm Hg. When the chest was opened a resistance to expiration was provided by placing the expiratory outlet from the respiratory pump under 3 cm of water.

The left side of the chest was opened in the fifth intercostal space and a small balloon placed in each of three pulmonary veins. Details of the technique have been described previously (LEDSOME and LINDEN, 1964). After the balloons had been placed in the pulmonary vein-atrial junctions the whole of the left lung root was completely occluded by means of ligatures. Thus only the right lung was either perfused or ventilated. The balloon catheters were led out of the chest and clamped. The chest was closed in layers, air expelled through a drainage tube inserted into a lower intercostal space and spontaneous respiration restored. Femoral arterial pressure was recorded through a metal cannula (Inconel, Johnson Matthey & Co., London, 1.5 mm bore) treated with a solution of dialkyl dimethylammonium chlorides (Arquad; Armour Hess, Ltd.) as a non-wetting agent. To the cannula was attached a Statham strain gauge (Model P23 Gb) the output of which was connected to a carrier amplifier (S.E. Laboratories; Feltham, Middlesex) and the pressure was recorded on a direct writing ultraviolet light recorder (S.E. Laboratories). The frequency response of the system obtained by the method of LINDEN (1959) was flat ($\pm 5\%$) to better than 60 c/s. The electrocardiogram was recorded from the right foreleg and left hindleg. The oesophageal temperature was maintained at 37.5 °C (± 1 °C) by adjusting a heater beneath the animal.

The tracheal cannula was connected by a single tube to a Y-piece in the limbs of which were placed light inspiratory and expiratory valves. The bore of the single tube was comparable to that of the trachea and its length was approximately that from the opening in the trachea to the tip of the nose; this arrangement compensated for loss of dead space produced by the tracheal cannulation. Respiration was recorded by connecting the valves to a closed circuit consisting of a light oxygen filled spirometer (BERNSTEIN and MENDEL, 1953) and a cylinder containing soda-lime. The counterweight of the spirometer was drilled and the core of an inductance transducer (S.E. Laboratories) tapped into its lower end. The inductance transducer was connected to a carrier amplifier (S.E. Laboratories) and movements of the spirometer were recorded on the ultraviolet light recorder. From the record so obtained respiratory rate, tidal volume and oxygen consumption were measured.

Samples of arterial blood were obtained anaerobically from the femoral artery at intervals throughout the experiment and carbon dioxide tension measured using a P_{CO_2} electrode (Electronic Inst. Ltd.); details of the technique have been described

previously (NORMAN, LEDSOME and LINDEN, 1965). In six experiments an infra-red carbon dioxide analyser (HARTMAN and BRAUN, URAS 4) was used continuously to measure the carbon dioxide percentage in the air from the tracheal cannula. Air was sampled at a rate of 30 l/hr and returned to the spirometer circuit. Calibration with gas mixtures of carbon dioxide in oxygen gave results which were reproducible to within $\pm 0.1\%$ CO_2.

Twelve animals were successfully prepared in which regular spontaneous respiration was resumed on disconnecting the respiration pump. In two animals there was no change in heart rate during distension of the pulmonary vein-atrial junctions. The results from these two experiments, therefore, have been excluded since there was no indication that any effective stimulus had been applied.

Results

In the ten dogs, which were breathing spontaneously using only one lung, the average minute ventilation was 316 ml/kg body wt/min (range 296–411), the average respiratory rate was 26 breaths/min (range 10–48), the average tidal volume was 10.6 ml/k-body wt/breath (range 7.5–17.8) and the average oxygen consumption was 10.8 ml/kg body wt/min (range 8.1–18.1). The values may be compared with those of another series of 10 dogs similarly anaesthetized and breathing spontaneously but without thoracotomy. The latter group had a similar oxygen consumption of 10.3 ml/kg/min but had a tidal volume of 15.4 ml/kg and a respiratory rate of 14 breaths/min. Thus by comparison the animals in the present experiments were breathing more rapidly and with a smaller tidal volume.

Measurements of heart rate and respiration were made during a control period of at least 1 min. The pulmonary vein-atrial junctions were then distended by injecting 1 ml of saline into each balloon; after 2 min, when a steady state had been reached, measurements were made for a further 1 min and the 1 ml of saline was then removed from each balloon. After another 2 min a second set of control measurements were made. Measurements made during distension of the balloons were compared with the average of the measurements during the two control periods. Forty-one distensions of the balloons in the pulmonary vein-atrial junctions were made in ten dogs. There were no persistent changes in either respiratory rate or tidal volume during any balloon distension although heart rate increased by an average of 18.4 beats/min (range 4–54). In five of the ten dogs there were transient changes in respiratory rate accompanied by a decrease in tidal volume occurring immediately after the balloons were distended and lasting 10–60 sec. Results will, therefore, be presented of quantitative measurements made after a steady state had been reached, and a qualitative description will be given of changes occurring transiently immediately following distension of the balloons.

THE EFFECTS OF DISTENSION OF THE PULMONARY VEIN-ATRIAL JUNCTIONS FOR 3 MIN

Measurements were made of heart rate, mean arterial pressure, respiratory rate and tidal volume in the control periods and during distension of the pulmonary vein bal-

TABLE 1

Effects of distension of balloons in the pulmonary vein-atrial junctions. All measurements were made over 1 min periods. Average results of 20 tests in ten dogs; ranges in brackets, ± S.E. of mean.

	Before distension	During distension (2nd to 3rd min)	After distension (2nd to 3rd min)	Change
Heart rate beats/min	110 (75–210) ±8.25	131 (92–216) ±7.37	116 (84–212) ±7.81	+ 18 (+4 to + 43) ±3.2
Mean B.P. mm Hg	140.3 (117–167) +3.3	139.7 (118–168) ±3.1	139.9 (119–162) ±3.0	−0.6 (−6 to +4.5) ±0.6
Respiratory rate breaths/min	26.8 (10–48) ±2.45	26.6 (9–48) ±2.5	26.0 (11–48) ±2.4	+ 0.46 (−3 to +4) ±0.38
Tidal volume ml (BTPS)	219 (115–338) ±15.3	225 (120–338) ±14.6	225 (125–338) ±14.2	+3.6 (−30 to +45) ±3.6

loons as described. The results of these measurements are presented in table 1. This table gives the averages of the results obtained in the first two balloon distensions made in each of ten dogs. This selection has been made to avoid overweighting the average values with results from those animals in which more tests were made. The changes in respiratory rate and tidal volume were not statistically significant. An example of the record from one of the experiments is shown in fig. 1. Distension of the pulmonary vein-atrial junctions by means of the small balloons caused an increase in heart rate but only small changes in respiratory rate and tidal volume. There were only small changes also in mean arterial blood pressure. In the five animals in which measurements of end-tidal carbon dioxide tension were made no changes were observed during the experimental periods once a steady state had been reached. The average end-tidal carbon dioxide tension was 39 mm Hg (range 37.5–42) before, during and after the experimental period. The maximum increase in end-tidal P_{CO_2} during the experimental period was 1 mm Hg and the maximum decrease was also 1 mm Hg. These changes are within the experimental error of the method of measurement. No measurements of arterial carbon dioxide tension were made during the experimental periods but in seventeen measurements of arterial P_{CO_2} made in the control periods the average difference between end-tidal and arterial carbon dioxide tension was 8 mm Hg (range 5.5–13). That the dogs were capable of increasing their ventilation was demonstrated in two of the dogs by changing the composition of inspired air from 100% O_2 to a

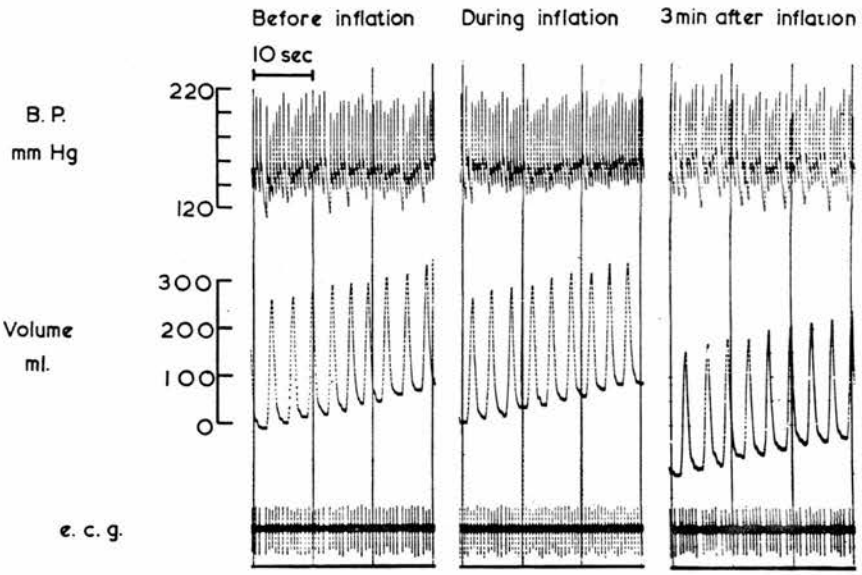

Fig. 1. Effects of distension of the pulmonary vein-atrial junctions; experiment illustrating a response typical of the average changes found. From above downwards femoral arterial pressure (mm Hg), spirometer record (ml) from which measurements of tidal volume, respiratory rate and oxygen consumption were made, electrocardiogram and a datum line. First column recorded immediately before inflating balloons in the pulmonary veins, second column after balloons had been inflated for 3 min, third column 3 min after removal of the inflation.

mixture of 5% carbon dioxide in oxygen: these dogs responded with an increase in tidal volume of 57 and 73 ml and increase in respiratory rate of 8 and 11 breaths/min.

An intravenous injection of propranolol (0.5 mg/kg "Inderal," I.C.I. Ltd.) was given to three dogs. Before injection of propranolol distension of the pulmonary vein-atrial junctions caused an average increase in heart rate of 19 beats/min (6 tests). In the same three dogs after injection of propranolol distension of the pulmonary vein-atrial junctions caused an average increase in heart rate of only 3 beats/min (6 tests). In a further three dogs in which distension of the pulmonary vein-atrial junctions caused an average increase in heart rate of 29 beats/min (6 tests) both vagus nerves were cut in the neck. After cutting the vagus nerves distension of the pulmonary vein-atrial junctions caused an average increase in heart rate of 2 beats/min (6 tests).

IMMEDIATE EFFECTS OF DISTENSION OF THE PULMONARY VEIN-ATRIAL JUNCTIONS

In three of the ten dogs there was a transient small increase in respiratory rate accompanied by a decrease in tidal volume which occurred immediately following the distension of the pulmonary veins and lasted 10–60 sec. An example is shown in fig. 2. The change in respiration was immediate, occurring on the first breath after distension of the balloons. The response was usually accompanied by a transient fall in arterial pressure of up to 20 mm Hg. The results in another such experiment are plotted in fig. 3. The fall in arterial pressure on balloon distension was usually accompanied by

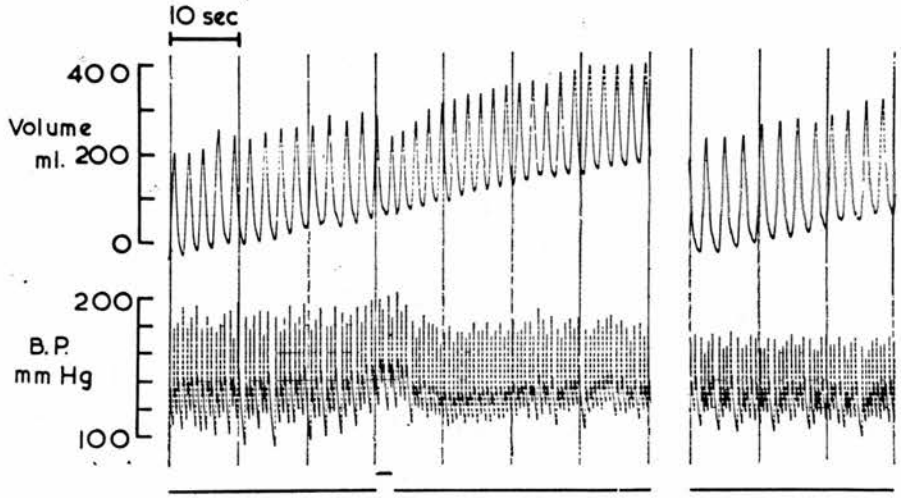

Fig. 2. Effects of distension of the pulmonary vein-atrial junctions. Conventions as in fig. 1. At the signal on the datum line the balloons in the pulmonary veins were distended. Second panel recorded 3 min later whilst distension was maintained. After 3 min heart rate remains 8 beats/min faster than in control period but respiration has slowed to less than control rate.

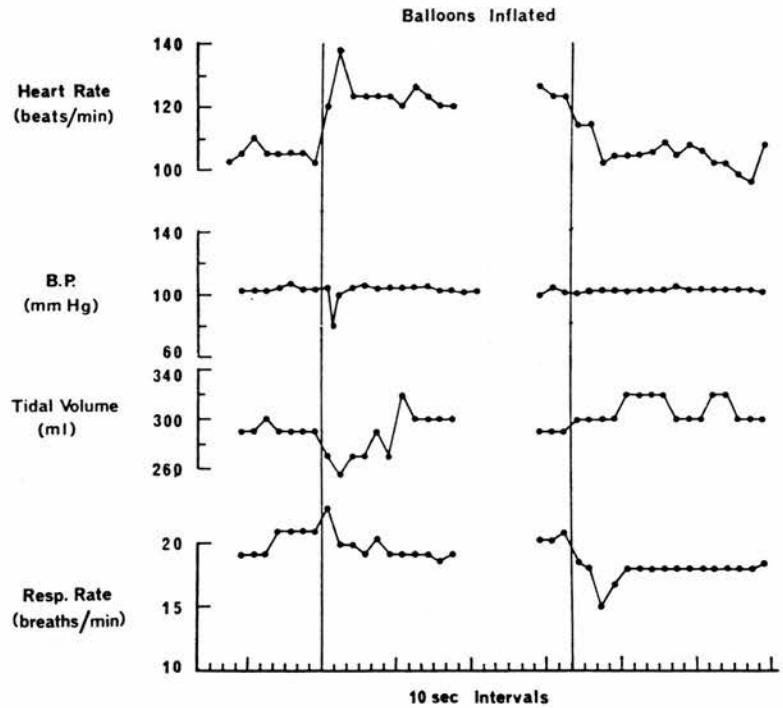

Fig. 3. Effects of distension of the pulmonary vein-atrial junctions. Measurements of heart rate, mean femoral arterial pressure, tidal volume and respiratory rate made over 10 sec periods and plotted to show changes. Balloons inflated in the pulmonary vein-atrial junctions during the period between the vertical lines. Heart rate remains elevated throughout this 3 min period, other variables show only transient changes.

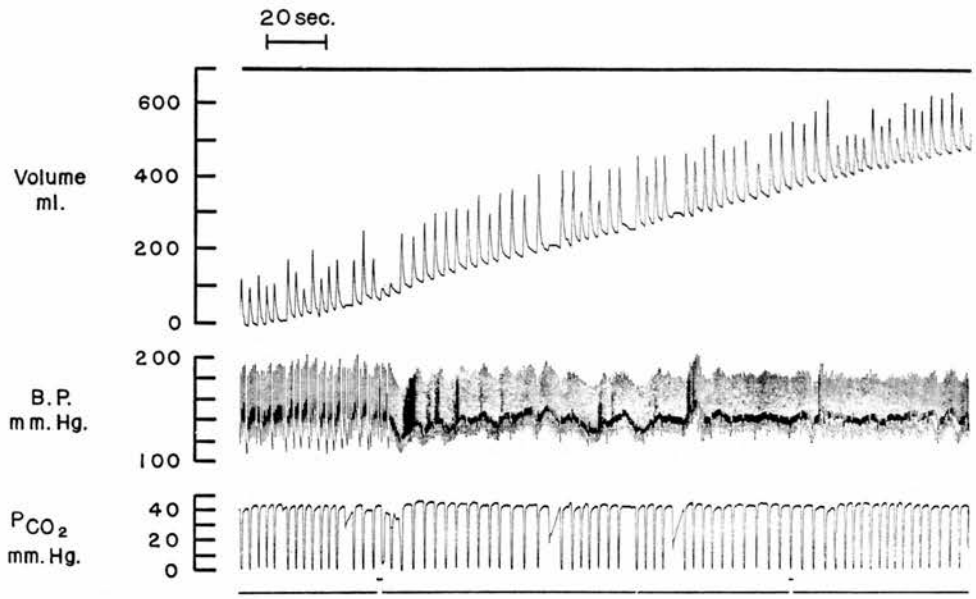

Fig. 4. Effects of distension of the pulmonary vein-atrial junctions. Conventions as in fig. 1. P_{CO_2} continuously sampled from tracheal cannula. At first signal mark on datum line balloons were inflated in pulmonary vein-atrial junctions, at second signal mark the inflation was removed.

an increase in heart rate but the presence or absence of a fall in arterial pressure made no difference to the steady state heart rate response. The transient changes in arterial pressure, heart rate and respiration were not affected by propranolol 0.5 mg/kg (2 dogs tested) which prevented the increase in heart rate described in the steady state. Cutting both vagus nerves in the neck did prevent the appearance of transient changes in respiration, heart rate or arterial pressure (2 dogs). In those dogs in which there was a fall in arterial pressure on balloon distension there was a transient increase of end-tidal P_{CO_2} of up to 3 mm Hg at the same time as the hypotension. The end-tidal P_{CO_2} then decreased to its previous value within 30 sec.

In two other dogs there was a very marked reduction in tidal volume for 2 or 3 breaths immediately after distension of the balloons. In these animals respiratory rate then slowed and tidal volume increased. An example of this type of response is shown in fig. 4.

In the other five dogs there were no changes in respiratory rate or tidal volume when the pulmonary vein balloons were distended.

Discussion

Several previous attempts have been made to relate changes of pressure in the heart and distension of the chambers of the heart to changes in respiration; such a mechanism might account for some of the changes in respiration associated with muscular exercise (COMROE, 1944). An increase in respiratory rate and tidal volume was reported

by HARRISON, HARRISON and MARSH (1932) in anaesthetized dogs during rapid intravenous infusions or during distension of the right atrium by means of a balloon. BOUCKAERT and PANNIER (1942) using two dogs with crossed circulations demonstrated that an increased venous inflow could cause an increase in the rate and depth of ventilation. In these experiments too many uncontrolled variables existed to allow conclusions to be made as to the mechanisms involved. A technique of separately perfusing the systemic and pulmonary circulations was used by DALY *et al.* (1937) who attributed a tachypnoea observed in some of their experiments to stimulation of receptors on the venous side of the pulmonary vascular bed. AVIADO *et al.* (1951) also described reflex tachypnoea which they attributed to stimulation of pulmonary venous receptors. More recently AVIADO and SCHMIDT (1959) have denied any effect of distension of the left atrium upon heart rate, vasomotor activity or respiration but did show respiratory inhibition as a result of distension of the left ventricle. In the majority of their experiments the stimulus was applied for only short periods of time (less than 30 sec) so that descriptions of changes in respiration may correspond to those described as transient in this paper.

In the present experiments in ten dogs distension of the pulmonary vein-atrial junctions always caused an increase in heart rate but caused no significant changes in respiratory rate or tidal volume. The response of an increase in heart rate was abolished or greatly reduced after cutting both vagus nerves in the neck or giving an intravenous injection of propranolol (0.5 mg/kg). Propranolol in this dose is known to reduce greatly the increase in heart rate resulting from stimulation of the cardiac sympathetic nerves (LEDSOME, LINDEN and NORMAN 1965). The results reported are entirely compatible with the conclusions of LEDSOME and LINDEN (1964), that stimulation of left atrial receptors results in an increase in heart rate by a reflex with an afferent path in the vagus nerves and an efferent path in the cardiac sympathetic nerves. In the present experiments an increase in heart rate on distension of the pulmonary vein-atrial junctions demonstrated that the stimulus was effective and that the afferent nervous pathway was intact.

The absence of a respiratory response is unlikely to have been due to the fact that the dogs were respiring on one lung and were not capable of changing respiration. The arterial carbon dioxide tension was not excessively raised nor were there any changes in carbon dioxide tension associated with balloon distension which may have affected the respiratory response. That the animals were capable of initiating changes in respiration was shown by the transient changes which occurred and by the fact that increasing the inspired concentration of carbon dioxide caused increased ventilation.

The mechanisms by which the transient changes in respiration were brought about are unknown but both respiratory inhibition (fig. 4) and tachypnoea with reduced tidal volume (fig. 3) were seen. Distension of the balloons in the pulmonary vein-atrial junctions causes an increase in atrial receptor discharge for as long as the distension is maintained (KIDD *et al.*, 1966). Since reflex increases in heart rate were observed which are maintained for as long as distension was continued (3 min) but were not accompanied by any maintained changes in respiration it is unlikely that stimulation

of left atrial receptors alone causes any change in respiratory rate or tidal volume.

It is possible that distension of the balloons caused temporary stimulation of receptors other than those in the pulmonary vein-atrial junctions in the present series of experiments, especially as the precise positions of the balloons were unknown. If there were excessive traction on the balloon catheters distension of the balloons could cause movement of the whole heart. Also injection of 3 ml of fluid into the balloons effectively increases the volume of the atrial contents by this volume and could lead to a temporary increase in ventricular volume so that immediately at the time of distension the stimulus may not have been limited to the pulmonary vein-atrial junctions.

It is concluded that distension of the pulmonary vein-atrial junction by means of small balloons causes a reflex increase in heart rate but no change in respiratory rate or tidal volume. Stimulation of left atrial receptors is, therefore, unlikely to contribute significantly to the hyperpnoea of muscular exercise.

Acknowledgements

The authors are indebted to Mr. R. HIRST for technical assistance and are grateful for support from a United States Public Health Service Research Grant No. TWOO234, the British Heart Foundation, the Medical Research Council and the Wellcome Trust.

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Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY,
VOL. LV, 1970

THE EFFECTS OF LEFT ATRIAL DISTENSION UPON URINE FLOW FROM THE ISOLATED PERFUSED KIDNEY.

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(*Received for publication 9th December* 1969)

An isolated kidney obtained from a donor dog was perfused at constant pressure with blood from the femoral artery of a recipient dog. The left atrial pressure of the recipient dog was increased by approximately 20 cm H_2O by inflating a balloon in the left atrium. Left atrial distension caused an increase in urine flow and sodium excretion from the isolated perfused kidney and on a few occasions from the dog's own kidneys. The results support the view that a blood borne agent is responsible for the diuretic response to left atrial distension.

Partial obstruction of the mitral orifice causes an increase of urine flow in anaesthetized [Henry, Gauer and Reeves, 1956; Ledsome, Linden and O'Connor, 1961] and unanaesthetized [Lydtin and Hamilton, 1964] dogs. Evidence has been presented supporting the view that stimulation of left atrial receptors is a major factor in the production of a diuretic response to left atrial distension [Ledsome and Linden, 1968]. However, the nature of the agent acting on the kidney to produce the diuresis has not yet been established [Gauer and Henry, 1963].

In the present investigation an isolated kidney from a donor dog was perfused at constant pressure with blood from a recipient dog, thus eliminating the possibility of changes in arterial pressure or activity in renal nerves affecting the kidney.

METHODS

Dogs weighing 14–33 kg were given a subcutaneous injection of morphine sulphate (0·5 mg/kg). One hour later under local anaesthesia (decicain, 2 per cent) a catheter was inserted through a saphenous vein into the inferior vena cava and each animal was anaesthetized by an intravenous infusion of 0·1 g/kg of chloralose (British Drug Houses) dissolved to make a solution of 1 g of chloralose in 100 ml. of sodium chloride solution (0·6 g/100 ml.). Subsequently during the experimental procedures a steady state of light anaesthesia and fluid input was maintained by the infusion every 10 min of 1·5 ml./kg of either sodium chloride solution (0·6 g/100 ml.) or the chloralose solution. As soon as possible after induction of anaesthesia artificial respiration was started with a mixture of 40 per cent oxygen in air, humidified at room temperature and supplied from a Starling 'Ideal' pump, the rate (about 18/min) and stroke (about 50 ml./3 kg body wt.) of which were adjusted approximately to equal that of the

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Printed in Great Britain

animal's spontaneous respiration. When the chest was opened a resistance to expiration was provided by placing the expiratory outlet from the respiratory pump under 2–3 cm of water.

The left side of the chest was opened in the fifth intercostal space and a balloon and a catheter (nylon, 1·5 mm bore) through which pressure was measured inserted into the left atrium through the appendage. This technique has been described previously [Ledsome, Linden and O'Connor, 1961]. Each ureter in the experimental animal was catheterized through a flank incision. A nylon cannula (internal diameter 1·5 mm, length 15 cm) was inserted into the right femoral artery, and the left femoral artery and vein were dissected free and cannulated.

A second dog was anaesthetized using the technique described. One kidney (usually the left) was exposed extraperitoneally through a flank incision. The ureter was cannulated and the renal artery and vein were dissected free. The renal artery was cannulated using a stainless steel cannula with an internal diameter of 3 mm and the renal vein was cannulated with a stainless steel cannula of internal diameter 6 mm. Both cannulae contained fine (0·5 mm bore) stainless steel tubes which led from outside the cannulae to the inside of the tips of the cannulae. Pressure was recorded from these fine tubes which were provided with lateral holes at their tips. After cannulation the kidney was removed from the donor dog and placed on a nest of warmed saline (NaCl 0·9 g/100 ml.) soaked swabs on the groin of the recipient animal.

Blood from the femoral artery of the recipient dog was mixed with an infusion of heparin (Heparin B.P. Mucous, 3 mg/min) and passed to a roller pump (M.H.R.E., Watson-Marlow Ltd) the rate of which was adjusted to maintain a constant blood level in a constant pressure cylinder. Air above the blood in the constant pressure cylinder was connected to a reservoir containing a large volume of air maintained at constant pressure. Blood flowed from the constant pressure cylinder through an electromagnetic flow probe into the renal arterial cannula. Blood flowing from the renal venous cannula was returned through the left femoral vein of the recipient dog. Blood flow to the perfused kidney was interrupted for a total of about 4–6 min in the course of cannulation. The total volume of blood contained in the external circuit was of the order of 90 ml.

Protamine sulphate B.P. was infused into the right femoral vein of the thoracotomized animal in a dose of 3·6 mg/min. This dose of protamine and that of the heparin was adequate to maintain a clotting time of greater than 60 min in the blood in the perfusion circuit and of 2–20 min in the systemic arterial blood. This technique of regional heparinization has been described previously [Carswell, Hainsworth and Ledsome, 1968].

Femoral arterial, renal arterial, renal venous and left atrial pressures were recorded from the appropriate cannulae connected to strain gauges (Statham Inst. Co. Model P23Gb). After amplification by means of carrier amplifiers (S.E. Laboratories, Feltham, Middlesex) the pressures were recorded on an ultra-violet light recorder (S.E. Laboratories). The manometers were calibrated in a stepwise manner using mercury and saline manometers. Zero pressure for each manometer was recorded *post-mortem* as pressure at the cannula tip with the tip free in air. Renal blood flow was recorded using an electromagnetic flowmeter (Statham Inst. Co. Medicon 4000); zero flow was checked by stopping flow for brief intervals throughout the experiment. Average variation in the zero flow reading during the course of an experiment was equivalent to a flow of 7 ml./min. The flowmeter was calibrated directly at the end of the experiment using blood from the recipient dog.

Urine was collected into test tubes from the thoracotomized dog's own kidneys and from the perfused kidney. Urine volume was measured every 10 min and the urine was analysed for sodium using a sodium electrode (Electronic Instruments Ltd, BH104 glass). The electrode was calibrated with gravimetrically prepared sodium chloride solutions covering the range 1–400 mM. The results obtained by this method for urinary sodium are slightly lower than those obtained by flame photometer

[Moore and Wilson, 1963). However, changes in urinary sodium concentration are reliably indicated by the electrode. Repeated estimations upon the 100 mM sodium chloride standard solution used throughout the experiments agreed to within $\pm 2{\cdot}6$ mM.

Body temperature of the recipient animal was maintained constant $\pm 1{\cdot}5$°C by adjusting heating lamps above and beneath the animal. The temperature of the perfused kidney indicated by a thermistor probe placed beneath the kidney was within 2°C of the dog's body temperature.

At intervals samples of arterial blood were withdrawn anaerobically from the renal arterial cannula. The blood was analysed for pH, P_{CO_2} and P_{O_2} and haematocrit using the techniques described by Norman, Ledsome and Linden [1965].

During the establishment of the perfusion circuit the dog was transfused with about 200–500 ml. of blood obtained from the dog which donated the kidney. At this time pH of the arterial blood was measured and brought to 7·38 units by infusion of sodium bicarbonate (1 M). After a period of 40 min or longer when urine flow from the perfused kidney had become steady, urine samples were collected for at least three 10 min periods. Provided the urine flow was reasonably constant at this time the left atrial balloon was distended with NaCl solution (0·9 g/100 ml.) until the left atrial pressure had increased by 20 cm H_2O. About 1 ml./kg body weight was usually required to produce the rise in left atrial pressure. The balloon was kept distended usually for 30 min but occasionally for longer. Urine samples were collected over at least four further 10 min periods before any subsequent intervention was made.

Results

The left atrium was distended by inflation of a balloon in 14 dogs. In 18 tests the average mean arterial pressure during the 30 min before inflation of the balloon in the left atrium was 111 mm Hg; this decreased to 99 mm Hg for the 30 min during which the balloon was inflated, and increased to 106 mm Hg for the 30 min immediately after the balloon was deflated. Heart rate increased from an average of 146 beats/min before balloon inflation to 173 beats/min during inflation and decreased to 151 beats/min after deflation. The average increase in mean left atrial pressure caused by balloon inflation was 20 cm H_2O. These changes were similar to those previously described [Ledsome *et al.*, 1961].

Urinary response from the isolated perfused kidney. Inflation of a balloon in the left atrium caused an increase in urine flow from the isolated perfused kidney in 18 tests in 14 dogs. The results are plotted in Fig. 1 in the form used previously [Ledsome *et al.*, 1961]; the mean rate of urine flow (ml./min) for the 30 min preceding atrial distension and the 30 min following the diuresis was regarded as the control rate, to be compared with the mean rate during the diuresis (i.e. the last 20 min of atrial distension and the first 10 min after removal of the distension). The average urine flow during the control periods was 0·4 ml./min and increased to 0·63 ml./min during the diuresis (57 per cent increase). The paired differences between the individual control and diuretic values were statistically significant ($P<0{\cdot}01$). Flow of urine usually increased gradually during the 30 min of balloon inflation but in some tests an increase in urine flow was observed during the first 10 min of balloon inflation. The largest urine flow sometimes occurred during the first 10 min after deflation of the balloon. This time course was similar to that previously described for the response of the intact kidney to left atrial distension.

The increase in urine flow caused by inflation of a balloon in the left atrium was generally (12 out of 18 tests) accompanied by a smaller increase in sodium excretion. The range of sodium excretion from the isolated perfused kidneys was from 1·2 μ-mole/min to 180 μ-mole/min. The average sodium excretion during the control periods was 24·6 μ-mole/min and increased to 33·9 μ-mole/min during the diuresis (38 per cent increase). The difference between the

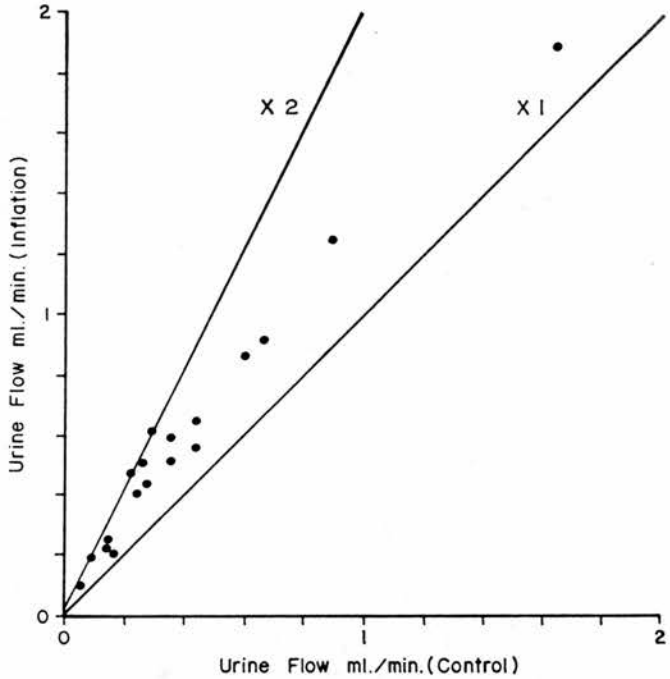

FIG. 1. Effects of distension of the left atrium upon urine flow from an isolated perfused kidney. Urine flow during inflation of a balloon in the left atrium compared with urine flow during the control periods (see text). Eighteen tests in 14 dogs. The line of no change is indicated by X1; that of a doubling of urine flow by X2.

average values was not significant. However, the paired differences between the individual control and diuretic values were significant ($p<0·05$) and there was a significant ($p<0·01$) positive correlation between a high increase in sodium excretion and a high increase in urine volume (Fig. 2). The range of sodium concentration in the urine was from 3·3 to 126 mM. During the diuretic response sodium concentration usually decreased; the average sodium concentration during the control periods was 51·9 mM and decreased to 45·8 mM during the diuresis. In three of the 18 tests sodium concentration increased during the diuresis leading to a more marked increase in sodium excretion. The changes observed in one of these three tests are shown in Fig. 3.

Left atrial distension did not invariably produce an increase in urine flow which could be distinguished from variations in urine flow in the control periods. On three occasions in three dogs left atrial distension failed to cause an increase in urine flow and urine was collected over the usual experimental periods. On

other occasions when urine flow was low and decreasing a transfusion of blood or dextran was given before completion of the experimental period. Thus a statistical analysis of the effects of atrial distension on every occasion it was performed was not possible. The three tests in which no response occurred are therefore not included in Fig. 1. However, an obvious increase in urine flow in response to atrial distension was demonstrated on at least one occasion in all 14 dogs in which such distension was attempted.

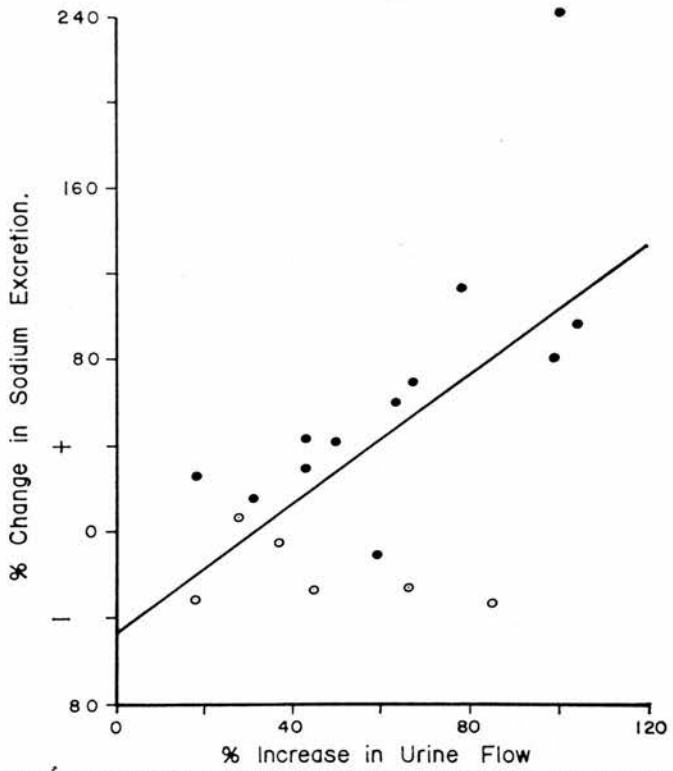

FIG. 2. Effects of distension of the left atrium upon urine flow and sodium excretion from the isolated perfused kidneys. Closed circles are those responses which were not accompanied by a diuresis from the dog's own kidneys; open circles are responses which were accompanied by a diuresis from the dog's kidneys. The calculated regression line is shown.

The urinary response in the dog's own kidneys. In most of the experiments the urine flow from the dog's own kidneys decreased and eventually became zero after perfusion of the isolated kidney had started. On five occasions in three dogs there was a small increase in urine flow from the dog's own kidneys in response to left atrial distension. The average urine flow during the control periods was 0·79 ml./min and increased to 1·01 ml./min during the diuresis (means from both kidneys of the dog). There was a smaller increase in sodium excretion of from 46 μ-mole/min during the control periods to 49 μ-mole/min during the diuresis. The changes observed in one test are shown in Fig. 4 in which the response in the dog's own kidneys may be compared with the simultaneous response from the perfused kidney. The time course of the changes in

urine volume and sodium concentration from the dog's own kidneys and from the perfused kidney were qualitatively similar in each of the five tests. The smaller increase in sodium excretion observed from the dog's own kidneys compared with the previous group was also seen in the urinary excretion from the perfused kidney in these tests. In these five tests urine flow from the perfused kidney increased from 0·37 ml./min in the control periods to 0·56 ml./

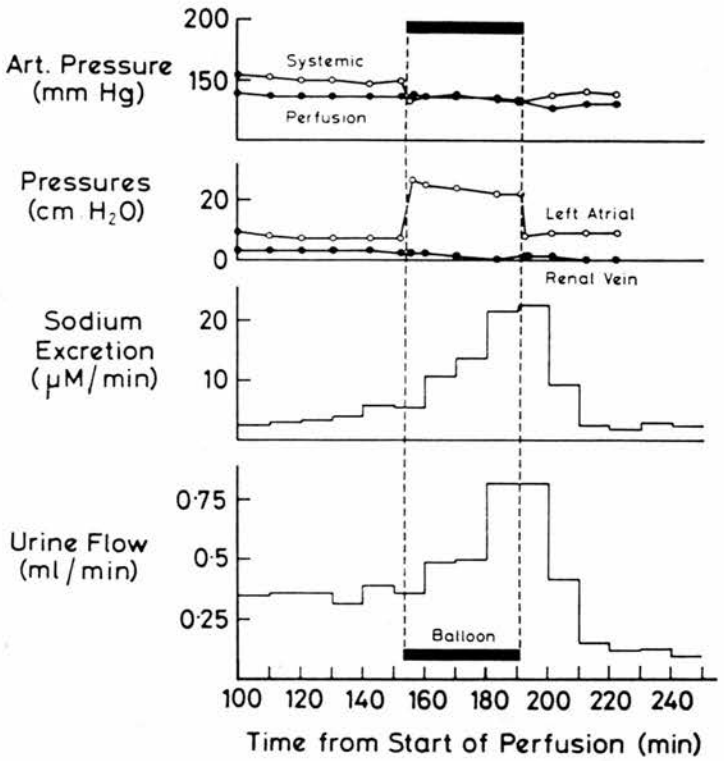

FIG. 3. Changes in sodium excretion and urine flow from an isolated perfused kidney during left atrial distension. Changes in systemic arterial pressure, renal perfusion pressure, left atrial pressure and renal venous pressure are also shown.

min during the diuresis; sodium excretion changed from 12·4 μ-mole/min to 11·7 μ-mole/min. Individual changes from the perfused kidneys in these five tests and from one other test in which the dog's own kidneys were producing urine are identified in Fig. 2. Urine flow from the dog's own kidneys in this sixth experiment was not included as a diuretic response because of a large change in the control urine flow values.

Blood flow to the perfused kidney. Each kidney was perfused at a constant pressure throughout each test of atrial distension. The average perfusion pressure used was 132 mm Hg (range 98–176 mm Hg); the mean renal perfusion pressure before balloon inflation was 132 mm Hg, 133 mm Hg during inflation and 132 mm Hg after inflation. The average pressure in the renal vein was 11 cm H_2O before inflation of the balloon, 10 cm H_2O during inflation and

9 cm H_2O after deflation. Total blood flow to the perfused kidney was measured in 14 tests of left atrial distension. The average blood flow before inflation of the balloon was 146 ml./min, during inflation of the balloon the flow was 132 ml./min and after deflation of the balloon it was 128 ml./min. The

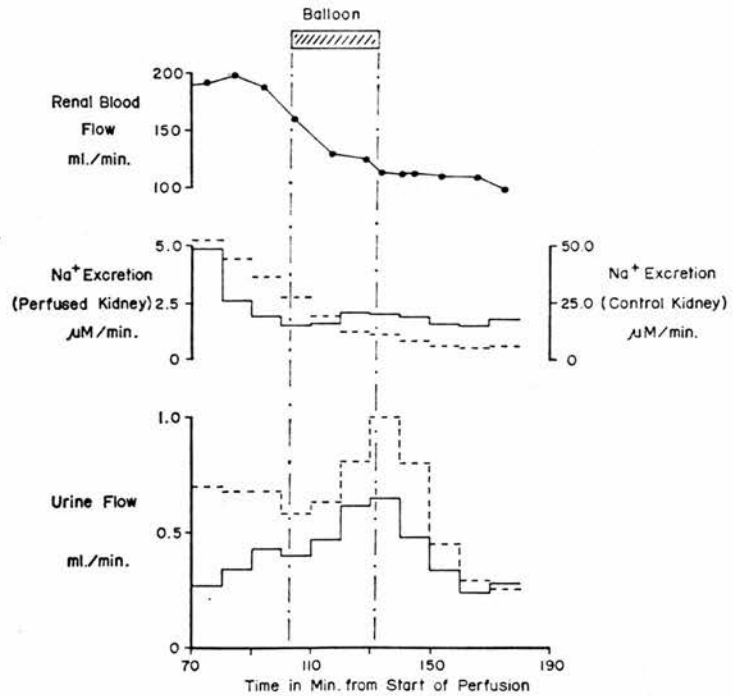

FIG. 4. Effects of left atrial distension on urine flow and sodium excretion from the perfused kidney (——) and both control kidneys (– – –) in one experiment. The blood flow to the perfused kidney is also shown. Kidney weight was 65 g. A balloon was distended in the left atrium during the period shown.

TABLE I. *Effect of inflation of a balloon in the left atrium upon urine volume, sodium excretion and the composition of arterial blood*

Average results in four tests. Figures for arterial blood analysis represent estimations on at least two separate samples before, during and after left atrial distension.

	Before inflation	During inflation	After inflation
Urine volume (ml./min)	0·33	0·50	0·32
sodium excretion (μ-mole/min)	20·4	18·5	11·2
Renal arterial blood analysis			
pH	7·28	7·26	7·24
P_{CO_2} mm Hg	36·0	34·0	35·0
P_{O_2} mm Hg	206·0	200·0	170·0
Haematocrit (% R.B.C.)	43·1	43·2	42·7

range of blood flow recorded was from 42–340 ml./min; average weight of the perfused kidneys was 59·2 g (range 42·7–83·5) thus the blood flow during the tests was usually of the order of 2 ml./g of kidney. Apart from a gradual decrease in blood flow which continued throughout the experiment there were no consistent changes in renal blood flow associated with distension of a balloon in the left atrium.

In four dogs at least two samples of blood were obtained from the renal artery of the perfused kidney in the 30 min before inflation of the balloon, in the 30 min whilst the balloon was inflated and in the 30 min after deflation of the balloon. The pH, P_{CO_2}, P_{O_2} and haematocrit were determined in each of these samples. The results obtained are listed in Table I. There was no consistent change in any of these variables which could be correlated with the changes in urine flow or sodium excretion which were observed.

Discussion

In the experiments described the only connection between the animal in which the left atrium was distended and the perfused kidney was the perfusing blood. The changes in urine flow and sodium excretion were therefore due to the action upon the kidney of a blood borne agent.

Previous work [Henry *et al.*, 1956; Ledsome *et al.*, 1961] showed that the diuresis occurring in response to left atrial distension was usually accompanied by a decrease in the concentration of sodium in the urine and thus by relatively small changes in sodium excretion. In a few experiments described by Arndt *et al.* [1963] and in all of the experiments of Lydtin and Hamilton [1964] there was a significant increase in sodium excretion in response to left atrial distension. In the experiments of Lydtin and Hamilton [1964] the increase in sodium excretion could be accounted for as secondary to the increase in arterial pressure that occurred. Arndt *et al.* [1963] measured clearances of para-aminohippuric acid and inulin which indicated increases in renal blood flow and glomerular filtration rate; they suggested that possibly two factors were involved in the production of a diuretic response, a decrease of antidiuretic activity in the circulating blood and an independent haemodynamic mechanism. There is a growing body of evidence which suggests that changes in plasma antidiuretic activity occur in association with stimulation of vascular receptors [Share, 1968]. It has yet to be demonstrated that such changes in plasma antidiuretic activity affect urinary excretion in these animals. It is difficult to reconcile these observations with the finding that infusion of vasopressin does not prevent the appearance of the diuretic response [Ledsome, *et al.*, 1961; Lydtin and Hamilton, 1964]. If more than one mechanism were involved then vasopressin might reduce without completely preventing the diuretic response; no information has yet been presented on this point. In the present experiments the pattern of urinary excretion observed – an increase in sodium excretion – was unlike an effect normally expected from a decrease in the release of antidiuretic hormone from the neurohyophysis. The significant correlation between the percentage increase in urine volume and the percentage increase in sodium

excretion in the diuresis does not support the concepts of separate diuretic and natriuretic agents.

The diuretic response to left atrial distension occurred in an isolated kidney perfused at constant pressure and in which there were no significant changes in total renal blood flow. Thus there was no evidence of a haemodynamic change in the kidney and no possibility of a change in renal perfusion pressure affecting sodium excretion. However, since an increase in renal medullary blood flow could account for the observed changes [Thurau, Deetjen and Kramer, 1960] and would represent only a small fraction of the total renal blood flow, the possibility of haemodynamic changes playing a part in the diuretic response is not entirely eliminated. If such changes did occur in the isolated perfused kidney then the agent acting on the kidney to produce the haemodynamic change must have been blood borne.

Mitral obstruction causes an increase not only of left atrial pressure but also of pressures throughout the pulmonary vascular bed. It seemed possible that in animals artificially ventilated at constant rate and volume, changes in acid-base balance of the arterial blood may have occurred. The measurements made showed only small changes in pH and P_{CO_2} during left atrial distension. The gradual fall in P_{O_2} was probably due to atelectasis and could be reversed by hyperinflating the lungs when the experimental period was over. This was not done during the experimental period to avoid introducing another complicating change. There were also only small variations in the haematocrit of the arterial blood. Thus it is unlikely that the diuretic response to left atrial distension was due to change in pH, P_{CO_2}, P_{O_2} or haematocrit of the arterial blood.

In those tests in which a diuretic response was observed from the dog's own kidneys the time course of the diuresis and the changes in sodium excretion were similar in the intact and the perfused kidneys. A diuretic response was observed from the intact kidneys in only five of the 18 tests; in the other tests urine flow was either inconstant, absent or so low as to be not measurable. The reason for the low urine flow from the dog's own kidneys is unknown but it may have been due to renal vasoconstriction or low perfusion pressure either before or during left atrial distension.

It was interesting that in those experiments in which the dog's own kidneys were functioning there were only decreases or very small increases in sodium excretion either from the intact or perfused kidneys. This raises the possibility that an agent promoting sodium retention could be released from the intact kidneys and might alter the characteristics of the diuretic response. However, the number of experiments was insufficient to assess the significance of this observation. The similarity of the responses when they did occur suggests that changes in renal arterial pressure and renal nervous activity do not greatly modify the characteristics of the response when it occurs. In a previous series no differences were observed between the changes in urine volume and sodium excretion in the innervated and denervated kidneys of the same dog [Ledsome *et al.*, 1961]. There must always be some doubt as to the adequacy of denervation when the vessels remain intact and the present observations confirm the view that changes in renal nervous activity do not contribute to the diuretic

response to left atrial distension. It is likely that the same blood borne agent was responsible for the diuretic response from both the perfused and the *in situ* kidneys.

The present experiments do not provide any further information on the nature of the agent acting on the kidney. They do allow the conclusion that during left atrial distension a blood borne agent causes an increase in urine flow and sometimes an increase in sodium excretion without causing significant changes in total renal blood flow.

Acknowledgments

The authors are indebted to Mr R. Hirst and Miss H. Newton for technical assistance and are grateful for support from a United States Public Health Service Research Grant No. TWO0234, the British Heart Foundation, the Medical Research Council and the Wellcome Trust.

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Canadian Journal of Physiology and Pharmacology

Published by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 49 NOVEMBER 1971 NUMBER 11

The Effects of Changes in the Rate of Infusion of Vasopressin in Anesthetized Dogs[1]

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Received February 9, 1971

MASON, J. M., and LEDSOME, J. R. 1971. The effects of changes in the rate of infusion of vasopressin in anesthetized dogs. Can. J. Physiol. Pharmacol. **49**, 933–940.

The dose rate of infusion of vasopressin which can be expected to provide maximal effects upon urine formation is difficult to predict in anesthetized dogs. The anesthetic agents, the state of hydration, and the osmolal excretion may all influence the effectiveness of vasopressin. The experiments demonstrate that in moderately hydrated anesthetized dogs when the rate of vasopressin infusion is changed from 0.4 to 0.04 mU/kg min^{-1} there is a transient dilution of the urine. The lower dose of vasopressin is four times that which completely inhibits water diuresis in conscious dogs and is larger than the dose of 0.025 mU/kg min^{-1} used in many experiments on anesthetized dogs to eliminate the effects of antidiuretic hormone on the kidney. It appears necessary in experiments in which an attempt is made to eliminate or to assess the effects of vasopressin upon a mechanism producing diuresis either to establish a maximum effective dose for the particular experimental cirmustances, or to examine the dose–response relationship between vasopressin and the diuresis in the particular experimental procedure.

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Chez le chien anesthésié, il est difficile de prévoir la dose et la vitesse d'infusion de vasopressine ayant un effet maximal sur la formation d'urine. L'agent anesthésiant, l'état d'hydratation et l'excrétion osmolaire sont autant des facteurs qui peuvent influencer l'action de la vasopressine. Ce travail démontre que chez le chien anesthésié et modérément hydraté, il y a une dilution transitoire de l'urine lorsque la vitesse d'infusion de vasopressine est diminuée de 0.4 à 0.04 mU/kg min^{-1}. La plus petite dose de vasopressine est encore quatre fois plus élevée que celle produisant une inhibition complète de la diurèse chez le chien éveillé. Cette dose est aussi plus grande que la dose (0.025 mU/kg min^{-1}) utilisée chez le chien anesthésié pour éliminer l'effet sur le rein d'hormones antidiurétiques. Il semble donc nécessaire, dans le but d'éliminer ou d'étudier les effets de la vasopressine sur les mécanismes de la diurèse, de déterminer la dose optimale de vasopressine compte tenu des besoins expérimentaux ou d'examiner la courbe dose–effet entre la vasopressine et la diurèse.

Introduction

The effectiveness of an infusion of vasopressin in reducing urine flow was investigated in conscious dogs undergoing water diuresis by Shannon (1942) and Verney (1947). Their results indicated that vasopressin infused at a rate of 0.01 mU/kg min^{-1} had a maximum effect in reducing urine flow. Unfortunately, assessment of the effectiveness of vasopressin is difficult in anesthetized dogs because water given intravenously or by stomach tube does not always produce diuresis (Verney 1929). However, Perlmutt (1961) obtained a dilute diuresis in severely hydrated anesthetized dogs and found that a dose of vasopressin of ap-

[1]This work was supported by a grant from the Medical Research Council of Canada.

proximately 0.07 mU/kg min^{-1} had a maximum effect in reducing urine flow although even higher doses did not produce maximum urine concentration. The differences in the effectiveness of vasopressin demonstrated in these studies have been attributed to the level of hydration (Perlmutt 1962). Other investigators have demonstrated a relationship between hydration, osmolal clearance, and alterations in the effective dose of vasopressin (Epstein *et al.* 1957; Levinsky *et al.* 1959; Orloff *et al.* 1957).

In a number of studies on mechanisms producing diuresis in anesthetized hydrated animals, a dose of vasopressin of 0.025 mU/kg min^{-1} or less has been used as adequate to ensure maximum antidiuresis (Mills *et al.* 1961; De Wardener *et al.* 1961; Ledsome *et al.* 1961; Lydtin and Hamilton 1964). The latter two groups of workers were examining the diuretic response to left atrial distension and their results have been interpreted as indicating that the diuretic response was unlikely to be due to changes in the rate of release of antidiuretic hormone from the neurohypophysis.

Experiments were designed to test the renal response to changes in the rate of vasopressin infusion at dose rates above 0.025 mU/kg min^{-1} and to examine the similarity of any diuretic responses to the diuretic response to left atrial distension. The results indicate that at the level of hydration and anesthesia described, a decrease from a high rate of vasopressin infusion to a rate greater than 0.025 mU/kg min−1 may cause a transient dilution of the urine.

Methods

Dogs of 12–26 kg were given a subcutaneous injection of morphine sulfate (0.5 mg/kg). One hour later under local anesthesia (mepivaccaine hydrochloride, 1%) a catheter was inserted through a saphenous vein into the inferior vena cava and each animal was anesthetized by an intravenous infusion of 0.1 g of chloralose (British Drug Houses) per kilogram, dissolved to make a solution of 1 g of chloralose in 100 ml of sodium chloride solution (0.6 g/100 ml). Subsequently during the experimental procedures a steady state of light anesthesia and fluid input was maintained by the infusion every 10 min of 1.5 ml of either sodium chloride solution (0.6 g/100 ml) or the chloralose solution per kilogram of body weight. In addition each animal had received an intramuscular injection of 5 mg desoxycorticosterone acetate in sesame oil on each of the 2 days preceding the experiment.

As soon as possible after the induction of anesthesia, artificial respiration was started with 40% oxygen in air supplied from a respiration pump (Harvard Apparatus Co., model 614), the rate (about 18/min) and stroke (about 50 ml/3 kg body weight) of which were adjusted to approximately equal that of the animal's spontaneous respiration. At intervals during the procedures samples of arterial blood were taken and pH, P_{CO_2}, and P_{O_2} were measured using appropriate electrodes (Instrumentation Laboratories Inc., No. 113-S1). Adjustments were made to the respiratory pump or small infusions (10–20 meq) of sodium bicarbonate solution (1 *M*) were given to maintain P_{CO_2} between 35 and 40 mm Hg and pH within the range 7.3–7.4. No adjustments were made during the control or experimental periods.

Each ureter was catheterized through a flank incision and urine volume was measured every 10 min. Femoral arterial pressure was recorded through a metal cannula (Inconel, 1.5 mm bore; Johnson, Matthey & Co., London). Central venous pressure was recorded through a 15-cm length of teflon tubing placed in the superior vena cava through the right external jugular vein. Zero pressure was determined *post mortem* as the level of the tip of the cannula free in air. To each cannula was attached a Statham strain gauge (model $P_{23}Gb$) and after amplification by means of a direct current amplifier (Honeywell; Accudata 113) the pressure was recorded on an ultraviolet light recorder (Honeywell; Visicorder 1508). The frequency response of the system recording femoral arterial pressure, obtained by the method of Hansen (1949), was flat (±5%) to better than 35 cycles per second. Mean pressures were obtained electrically. Values were recorded every 10 min at the midpoint of the urine collection period.

During the surgical procedures, about 1 h, the animals received a slow infusion of 100 ml dextran (6% dextran 75 in 0.9% sodium chloride; Travenol Laboratories Inc.) for each 13 kg body weight (approximately 10% of their blood volume). The electrocardiogram was recorded from leads on the forelegs and chest wall; heart rates were counted from the electrocardiogram over periods of at least 30 s.

Infusions of vasopressin (Pitressin, batch No. LY129H, containing 10 pressor units per milliliter; Parke Davis & Co. Ltd.) were given through a catheter inserted into a femoral vein. The vasopressin was diluted to the required strength in 0.6% (w/v) sodium chloride solution and delivered by a motor driven syringe pump (Harvard Apparatus Co. Inc.) at a rate of 1.0 ml/min. Inulin was infused at a rate of 1.0 ml/min by a motor-driven syringe pump (Harvard Apparatus Co. Inc.) through a cannula inserted into the left external jugular vein. The inulin was dissolved in 0.6% (w/v) sodium chloride solution in an amount calculated from Smith (1956) to maintain plasma inulin levels at 25 mg/100 ml. Esophageal temperature was maintained at 37 ± 1.5 °C using a heating pad and temperature controller (Yellow Springs Instrument Co.).

Samples of 4 ml of arterial blood were taken every 20 min into syringes moistened with heparin and the

TABLE 1. Effects of changing the rate of vasopressin infusion upon heart rate and blood pressure

	One-half hour infusion			Two hour infusion		
	Control		Experimental	Control		Experimental
Heart rate, beats/min*	120±10.1	$n = 21$ $d = 12$ $t = 5.4$ $2P < 0.001$	132±10.3	83±13.5	$n = 6$ $d = 7$ $t = 2.1$ $2P < 0.10$	90±15.8
Blood pressure,* mm Hg	150±4.6	$d = 5$ $t = 4.2$ $2P < 0.001$	145±4.5	143±4.4	$d = 6$ $t = 1.5$ $2P < 0.20$	137±4.8

*Means ± S.E.M.

blood was centrifuged immediately; the volume removed was replaced with dextran. Urine and plasma were analyzed for sodium and potassium using a flame photometer (Instrumentation Laboratories Inc., model 143). Urine and plasma osmolality was measured by freezing point depression (Osmette; Precision Systems). Urine and plasma were assayed for inulin by the method of Roe *et al.* (1949). Duplicate determinations of inulin concentrations agreed to within ±5%.

Experimental Protocol

One group of eight dogs received an infusion of vasopressin at a rate of 0.4 mU/kg min^{-1} for 90 min. The concentration of vasopressin being infused was then reduced, decreasing the rate of infusion to 0.4 mU/kg min^{-1} for a period of 30 min, after which infusion at the previous concentration was restored (½-h infusion experiments). The procedure was repeated at least twice and not more than three times with each dog. A second group of six dogs received alternating infusions of 0.4 and 0.04 mU/kg min^{-1} for 2 h at each rate (2-h infusion experiments). In these experiments the first infusion of vasopressin at a rate of 0.4 mU/kg min^{-1} was started 30 min after the surgical procedures were completed.

To allow analysis of the results, values of variables obtained during control periods were compared with those obtained during experimental periods using the technique of paired differences. In the ½-h infusion experiments the control values were the averages of the values in the last three 10-min periods during infusion of vasopressin at a rate of 0.4 mU/kg min^{-1} and the three 10-min periods immediately following the experimental period. The experimental values were the averages of the last two 10-min periods during infusion of vasopressin at a rate of 0.04 mU/kg min^{-1} and the first 10-min period immediately after resumption of infusion of vasopressin at a rate of 0.4 mU/kg min^{-1}. This analysis of the results was similar to that used previously in investigation of the diuretic response to atrial distension (Ledsome *et al.* 1961). In the 2-h infusion experiments the control values were the averages of the values in the last six 10-min periods during infusion of vasopressin at a rate of 0.4 mU/kg min^{-1} before and after infusion at the lower rate. The experimental values were the averages of the fourth to the ninth 10-min periods after beginning the infusion of vasopressin at a rate of 0.04 mU/kg min^{-1}. In two dogs in the 2-h experiments urine was collected from only one kidney. Measurements of urine flow and excretion in these two dogs were doubled to make the values comparable with those from the other dogs.

Results

Cardiovascular Effects

Changing the rate of vasopressin infusion from 0.4 to 0.04 mU/kg min^{-1} led to an increase in heart rate and a decrease in arterial pressure in both the ½-h and 2-h infusion experiments (Table 1). Qualitatively similar changes occurred in both series of experiments. The cardiovascular changes occurred within the first 10 min after the start of the infusion of vasopressin at a rate of 0.04 mU/kg min^{-1} and returned towards previous control levels with resumption of the infusion at a rate of 0.4 mU/kg min^{-1} (Fig. 1). Central venous pressure (11.0 cm H_2O, S.E.M. ±0.2) did not change in the four 2-h infusion experiments in which it was measured.

Urinary Effects

The urinary effects observed during the ½-h and 2-h infusion experiments are summarized in Table 2. Reduction in the rate of vasopressin infusion for a 30-min period caused a significant dilution of the urine and a significant increase in free water clearance. These changes were accompanied by decreases in osmolal clearance, sodium and potassium excretion, and a small but significant ($2P < 0.1$) increase in urine flow. The time course of the changes is shown in Fig. 1.

Qualitatively similar effects occurred in the 2-h infusion experiments. However, in the

TABLE 2. Effects of changing the rate of vasopressin infusion upon urinary excretion

	One-half hour infusion		Two hour infusion	
	Control	Experimental	Control	Experimental
	$n = 21$		$n = 6$	
Urine flow, ml/min*	1.26±0.12	1.48±0.20	0.96±0.23	2.00±0.74
	$d = 0.22$ $t = 1.95$ $2P < 0.10$		$d = 1.04$ $t = 1.94$ $2P < 0.20$	
Urine osmolality, mosmol/kg*	780±84	712±101	710±77	424±110
	$d = 68$ $t = 2.25$ $2P < 0.05$		$d = 287$ $t = 5.38$ $2P < 0.005$	
Osmolal clearance, ml/min*	2.73±0.23	2.54±0.22	1.95±0.33	1.59±0.18
	$d = 0.19$ $t = 2.07$ $2P < 0.10$		$d = 0.37$ $t = 2.33$ $2P < 0.10$	
Free water clearance, ml/min*	−1.46±0.20	−1.05±0.24	−1.00±0.12	+0.41±0.57
	$d = 0.41$ $t = 5.05$ $2P < 0.001$		$d = 1.41$ $t = 2.07$ $2P < 0.10$	
Sodium excretion, mmol/min*	0.19±0.00	0.17±0.00	0.16±0.03	0.12±0.01
	$d = 0.02$ $t = 3.17$ $2P < 0.005$		$d = 0.04$ $t = 2.45$ $2P < 0.10$	
Potassium excretion, mmol/min*	0.09±0.01	0.07±0.01	0.07±0.01	0.05±0.01
	$d = 0.02$ $t = 5.89$ $2P < 0.001$		$d = 0.02$ $t = 1.85$ $2P < 0.20$	
Glomerular filtration rate, ml/min*	100±10	100±10	86±22	82±15
	$d = 0$ $t = 0.3$ $2P$ n.s.		$d = 4$ $t = 0.7$ $2P$ n.s.	

*Means ± S.E.M.

2-h infusion experiments there was a quantitatively greater dilution of the urine with a quantitatively greater increase in urine flow and free water clearance. Decreases occurred in osmolal clearance and sodium excretion.

In the 2-h infusion experiments the changes in urine flow, urine osmolality, and free water clearance were transient. The changes reached a peak approximately 70 min after the start of the 0.04 mU/kg min^{-1} infusion of vasopressin and then the values began to return to previous control levels (Fig. 2). The changes in heart rate and arterial pressure were not transient. Changes in solute excretion were small and no characteristic pattern could be established (Fig. 2).

In the first hour of the 2-h infusion experiments there was a transient diuresis involving an increase in osmolal clearance which occurred about 1 h after completion of the surgical procedures. The appearance of this osmotic diuresis, which may have been secondary to the dextran infusion, was not confined to these six experiments but appeared also in the ½-h infusion experiments. However, in the latter experiments a period of approximately 1½ h elapsed between the surgical procedures and the first control period. The osmotic diuresis therefore does not appear in the analysis of the other experimental results.

No significant changes in glomerular filtration rate were measured by the method used in these experiments. The infusion of vasopressin and inulin had no effect on plasma osmolality

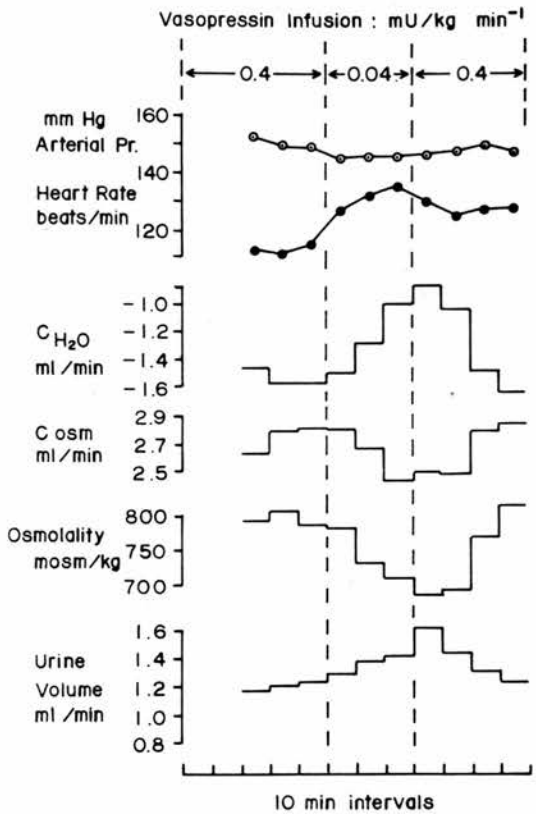

FIG. 1. Effects of changing the rate of vasopressin infusion for a 30-min period. Each horizontal line in a 10-min interval represents the average value from 21 tests in 8 dogs. From above downwards: femoral arterial pressure (mm Hg), heart rate (beats/min), free water clearance (ml/min), osmolal clearance (ml/min), urine osmolality (mosmol/kg), urine flow (ml/min). Dotted lines indicate change in the rate of vasopressin infusion.

or sodium and potassium concentrations. The average (± S.E.M.) plasma variables during the control and experimental periods in the 14 dogs were: sodium, 140 ± 5.47 mmol/l; potassium, 4 ± 0.53 mmol/l; osmolality, 294 ± 6.69 mosmol/kg.

Discussion

In the experiments described the assumption had been made that the experimental animals were releasing relatively constant amounts of endogenous antidiuretic hormone throughout the control and experimental periods. Changes in the measured variables would then be due to changes in the rate of vasopressin infusion.

Reduction in the rate of vasopressin infusion for a 30-min period was associated with dilution of the urine, a small increase in free water clearance, an increase in heart rate, and a decrease in arterial pressure. The decreased concentration of solutes without any large change in the rate of excretion of solutes and the time course of the changes were similar to the changes which occur during water diuresis. Changes in heart rate and arterial pressure occurred with the change in the rate of vasopressin infusion, indicating that these changes were also probably mediated by changes in the circulating vasopressin concentration. Significant cardiovascular changes in response to vasopressin infusion are usually considered to occur within a dose range higher than that in which urinary effects are observed. Rocha e Silva and Rosenberg (1969) infused vasopressin into anesthetized dogs at rates between 0.8 and 6.0 mU/kg min^{-1} and reported only a slight initial rise in blood pressure and a decrease in heart rate; these effects lasted for only 2–4 min. The small decrease in solute excretion observed in the present experiments may have been due to a decrease in glomerular filtration rate secondary to the cardiovascular effects of the vasopressin. The fact that no changes in glomerular filtration rate were measured may not be significant since the accuracy of the method of measurement of inulin clearance is not adequate to detect small changes in glomerular filtration rate that are capable of altering solute excretion.

Qualitatively similar changes in urinary excretion, heart rate, and arterial pressure occurred when the rate of infusion of vasopressin was reduced for a 2-h period. There was a quantitatively greater dilution of the urine and after 70 min the urine flow and free water clearance began to decrease and osmolality to increase. The characteristics of the diuresis associated with the 2-h infusions were compatible with the known actions of vasopressin. However, the time course of the diuresis was not as expected following removal and replacement of vasopressin in the blood. If the half-life of vasopressin in the blood is 5 min (Lauson and Bocanegra 1961) then a reduced stable concentration in the blood would be expected about 30 min after the change to the lower rate of infusion. Infusions of vasopressin during water diuresis have been shown to have

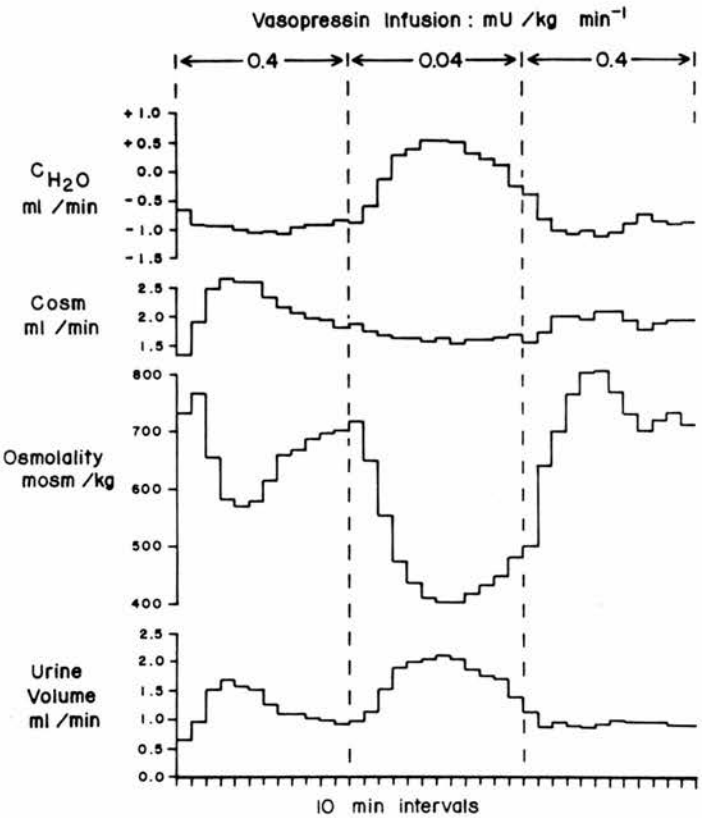

FIG. 2. Effects of changing the rate of vasopressin infusion for a 2-h period. Each horizontal line in a 10-min interval represents the average value from six tests in six dogs. From above downwards: free water clearance (ml/min), osmolal clearance (ml/min), urine osmolality (mosmol/kg), urine flow (ml/min). Dotted lines indicate change in the rate of vasopressin infusion.

maximum effects in about 30 min (Verney 1947) and in these experiments when vasopressin infusion was stopped urine flow returned to the diuretic level in about the same time. In the present experiments the urine flow and concentration continued to change over the whole 2-h period as shown in Fig. 2. It must be concluded that in the moderately hydrated anesthetized dog when the concentration of vasopressin in the blood is reduced from a high level to a lower level, there will be dilution of the urine which will be at least partially transient.

It is apparent from the present experiments in dogs anesthetized and hydrated in a similar fashion to those used by Ledsome *et al.* (1961) and Lydtin and Hamilton (1964) in experiments on left atrial distension, a dose of vasopressin of 0.025 mU/kg min^{-1} would not constitute a maximally effective dose if relatively large changes in vasopressin concentration in the blood occurred. The conclusion that the diuretic response to left atrial distension could not be due to a decrease in the rate of release of vasopressin from the neurohypophysis was therefore not justified.

It is of interest that the diuretic response to left atrial distension has been described as transient (Henry *et al.* 1956; Ledsome *et al.* 1961; Lydtin and Hamilton 1964), reaching a peak 50–90 min after the beginning of left atrial distension. This transient response has been observed despite continued distension of the left atrium and despite a continued reduction in blood antidiuretic activity (Shu'ayb *et al.* 1965, Figs. 2 and 3). The similarity in time course with the present experiments is obvious. However, the diuretic response to left atrial

distension is accompanied by an increase in solute excretion in addition to an increase in free water clearance (Ledsome *et al.* 1961; Lydtin and Hamilton 1964; Arndt *et al.* 1963), whereas in the present experiments a decrease in the rate of infusion of vasopressin was accompanied by an increase in free water clearance but a decrease in solute excretion. Thus the diuretic response to left atrial distension is unlikely to be totally accounted for by a reduction in the circulating levels of antidiuretic hormone.

The effectiveness of vasopressin in concentrating the urine has been shown to be altered by the degree of hydration of the animals (Perlmutt 1962) and by the osmolal clearance (Orloff *et al.* 1957). There is also some evidence that the sensitivity to exogenous vasopressin is increased when release of endogenous hormone is reduced, for example, after alcohol administration (Pickford 1966). Shannon (1942) had examined this possibility in conscious dogs undergoing water diuresis but was unable to find evidence to support such a change in sensitivity. Predictions of a dose of vasopressin which will cause a maximum reduction in urine flow or concentration may be valid only if tests are made with animals in a similar state of anesthesia, hydration, and osmolal excretion to the experimental animals. This may mean that inducing water diuresis and finding a dose of vasopressin which inhibits such a diuresis is not a suitable test for effective antidiuretic activity under other circumstances. The use of doses of vasopressin much in excess of those having effects on urine production may be undesirable because of the possibility of affecting the cardiovascular system. It is necessary in experiments in which an attempt is made to eliminate or assess the effects of vasopressin upon a mechanism producing diuresis either to establish a maximum effective antidiuretic dose for the particular experimental design or preferably to establish a dose–response relationship with different doses of vasopressin in the actual experimental procedure. It is unfortunate that such a maximum effective antidiuretic dose may be found to produce cardiovascular effects, thus complicating interpretation of the results.

We wish to thank Mrs. Glenda Bennion for her expert assistance.

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J. Physiol. (1972), **221**, *pp.* 427–440
With 3 text-figures
Printed in Great Britain

THE EFFECTS OF VASOPRESSIN ON THE DIURETIC RESPONSE TO LEFT ATRIAL DISTENSION

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(*Received 5 July 1971*)

SUMMARY

1. The diuretic response to left atrial distension was studied during infusion of saline or during infusion of vasopressin at four different dose rates.

2. During infusion of saline left atrial distension caused an increase in free water clearance and an increase in osmolal clearance.

3. During infusion of vasopressin at a rate of 0·025 m-u./kg.min and 0·1 m-u./kg.min there was no statistically significant increase in free water clearance during left atrial distension but there were variations in free water clearance in individual experiments.

4. During infusion of vasopressin in doses of 0·4 m-u./kg.min and 1·0 m-u./kg.min changes in free water clearance were dependent upon changes in osmolal clearance indicating that these doses of vasopressin provided a maximum antidiuretic effect.

5. Infusion of vasopressin had no effect on the increase in osmolal clearance which occurred in response to left atrial distension.

6. The results allow the view that a part of the diuretic response to left atrial distension could depend upon a decrease in the concentration of antidiuretic hormone in the circulating blood.

INTRODUCTION

Partial obstruction of the mitral orifice causes an increase in urine flow in anaesthetized (Henry, Gauer & Reeves, 1956) and unanaesthetized dogs (Lydtin & Hamilton, 1964). It has been suggested that the diuretic response is due at least in part to a decrease in the rate of release of antidiuretic hormone from the neurohypophysis (Arndt, Reineck & Gauer, 1963; Johnson, Moore & Segar, 1969) and it has been shown that left atrial distension is accompanied by a decrease in antidiuretic activity in the circulating blood (Shu'ayb, Moran & Zimmerman, 1965). However, the

diuretic response occurs during infusion of vasopressin adequate to completely inhibit water diuresis in the conscious dog (Ledsome, Linden & O'Connor, 1961; Lydtin & Hamilton, 1964). The part which antidiuretic hormone plays in the diuretic response to left atrial distension is therefore in doubt.

The present investigation was carried out to determine the effects of a range of doses of vasopressin upon the diuretic response to left atrial distension and thus provide more detailed information regarding the role of vasopressin in the diuretic response.

METHODS

Dogs of 12–26 kg were given a subcutaneous injection of morphine sulphate (0·5 mg/kg). One hour later under local anaesthesia (mepivacaine hydrochloride, 1 %) a catheter was inserted through a saphenous vein into the inferior vena cava and each animal was anaesthetized by an intravenous infusion of chloralose 0·1 g/kg (British Drug Houses), dissolved to make a solution of 1 g chloralose in 100 ml. sodium chloride solution (0·6 g/100 ml.). Subsequently during the experimental procedures a steady state of light anaesthesia and fluid input was maintained by the infusion every 10 min of 1·5 ml./kg body wt. of either sodium chloride solution (0·6 g/100 ml.) or the chloralose solution.

As soon as possible after the induction of anaesthesia artificial respiration was started with a mixture of 40 % oxygen in air, supplied from a respiration pump (Harvard Apparatus Co., model 614) the rate (about 18/min) and stroke (about 50 ml./3 kg body wt.) of which were adjusted to approximately equal that of the animal's spontaneous respiration. When the chest was opened a resistance to expiration equivalent to 3 cm H_2O was provided by an exhalation valve (Ohio Chemical). At intervals during the procedures samples of arterial blood were taken and pH, P_{CO_2}, and P_{O_2} measured using appropriate electrodes (Instrumentation Laboratories Inc. No. 113–S1). Adjustments were made to the respiratory pump or small infusions (10–20 m-equiv) of sodium bicarbonate solution (1 M) were given to maintain P_{a,CO_2} between 35 and 40 mm Hg and pH within the range 7·3–7·4; no adjustments were made during the control or experimental periods.

Each ureter was catheterized through a flank incision and urine volume was measured every 10 min. The left side of the chest was opened in the fifth intercostal space and a balloon placed in the left atrium as described previously (Ledsome *et al.* 1961).

Femoral arterial pressure was recorded through a metal cannula (Inconel; Johnson, Matthey & Co., London: 1·5 mm bore) and left atrial pressure through a 15 cm length of teflon tubing (1 mm bore). To each cannula was attached a Statham strain gauge (Model $P_{23}Gb$) and after amplification by means of a d.c. amplifier (Honeywell, Accudata 113) the pressure was recorded on an ultraviolet light recorder (Honeywell Visicorder 1508). The frequency response of the system recording femoral arterial pressure, obtained by the method of Hansen (1949), was flat (± 5 %) to better than 35 c/s. Mean pressures were obtained electrically.

During the surgical procedures, about 2 hr, the animals received a slow infusion of 100 ml. dextran (6 % dextran 75 in 0·9 % sodium chloride, Travenol Laboratories Inc.) for each 13 kg body wt. (approximately 10 % of their blood volume). The electrocardiogram was recorded from leads on the forelegs and chest wall; heart rates were counted from the electrocardiogram over periods of at least 30 sec.

Infusions of vasopressin (Pitressin, Parke Davis & Co. Ltd., Batch no. LY 129H, containing 10 pressor u./ml.) were given through a catheter inserted into a femoral vein. The vasopressin was diluted to the required strength in 0·6 % (w/v) sodium chloride solution and delivered by a motor driven syringe pump (Harvard Apparatus Co. Inc.) at a rate of 1 ml./min. Oesophageal temperature was maintained at 37° C ± 1·5° C using a heating pad and temperature controller (Yellow Springs Inst. Co.).

Samples of arterial blood (4 ml.) were taken every 20 min into syringes moistened with heparin and the blood was centrifuged immediately; the volume removed was replaced with dextran. Urine and plasma were analysed for sodium and potassium using a flame photometer (Instrumentation Laboratories Inc., model 143). Urine and plasma osmolality were measured by freezing point depression (Osmette, Precision Systems). The average difference between duplicate estimations was 4·4 m-osmole/kg.

Experimental protocol. In each animal three tests of atrial distension were carried out. One group of nine animals received infusions of sodium chloride 0·6 % (w/v), vasopressin 0·1 m-u./kg.min and vasopressin 1·0 m-u./kg.min; a second group of ten animals received infusions of sodium chloride 0·6 % (w/v), vasopressin 0·025 m-u./kg.min and vasopressin 0·4 m-u./kg.min; the infusions were given in a random order in each animal. After the surgical procedures were completed urine collection began. One hour later an infusion of either the sodium chloride solution or a vasopressin solution was started. After 50 min the left atrial balloon was distended with saline (about 1 ml./kg) to increase left atrial pressure by about 20 cm H_2O. Left atrial distension was maintained for 30 min and the infusion continued for 40 min after release of the distension. The rate of infusion of vasopressin was then changed and after a further 50 min a second distension was performed. The third distension was performed after similar time intervals. The control values were taken to be the averages of the values in the three 10 min periods preceding atrial distension and the three 10 min periods following the experimental periods. The experimental values were the averages of the last two 10 min periods during atrial distension and the first 10 min period immediately after release of the distension. Values during the control or experimental periods in each group were compared using the Student's t test for paired data; these values were compared between the two groups using the t test for unpaired data.

RESULTS

Cardiovascular effects of atrial distension and vasopressin infusion. Distension of a balloon in the left atrium caused a rise in left atrial pressure, a fall in mean arterial pressure and an increase in heart rate. The average changes during the experiments in which vasopressin was infused at different doses are shown in Table 1. Although heart rate appeared to be slower with high doses of vasopressin the values of left atrial pressure, mean arterial pressure and heart rate during the control periods in each of the five groups were not significantly different from one another nor were the values during the experimental periods significantly different from one another.

Changes in urinary composition during saline infusion. The effects of atrial distension observed during the infusion of saline serve as controls with which the effects of atrial distension during the infusion of vasopressin at four different dose rates may be compared. Distension of the left

atrium was usually associated with an increase in urine flow (Table 2). The results obtained during saline infusion in the two groups of dogs have been pooled since none of the observed variables showed any significant differences between the two groups of dogs. The time course of the changes in urine flow and other variables which occurred during atrial distension was similar to that previously reported (Ledsome *et al.* 1961) and is illustrated in Fig. 1 which summarizes the results of seventeen tests. The increase in urine flow usually began 5–15 min after starting left atrial distension and the maximum rate of urine flow was usually reached in the last period of atrial distension or the first period following removal of the distension. The increase in urine flow was usually accompanied by a marked decrease in urine osmolality, an increase in osmolal clearance and an increase in free water clearance (Table 2). The time course of these changes followed the changes in urine flow with the exception that osmolal clearance frequently increased in the first 10 min period following atrial distension and decreased rapidly following release of the atrial distension (Figs. 1, 2). Only small changes in sodium excretion and potassium excretion occurred in these animals (Table 2). The values in Table 2 and Fig. 1 indicate that

TABLE 1. Cardiovascular effects of left atrial distension and infusion of vasopressin: C control periods, E experimental periods

		Rate of infusion of vasopressin (m-u./kg.min)									
		Saline		0·025		0·1		0·4		1·0	
		C	E	C	E	C	E	C	E	C	E
	n	17		10		9		10		9	
Heart rate (beats/min)	$\bar{x}$	137	180	134	191	139	194	124	173	109	168
	± S.E. of mean	10·1	9·8	15·0	9·6	12·9	16	12·6	8·8	14·7	12·9
	$\bar{d}$	43		56·5		55·6		49·3		59	
	t	5·6		3·8		6·9		4·6		6·2	
	$2P$	< 0·001		< 0·005		< 0·001		< 0·001		< 0·001	
Mean B.P. (mm Hg)	$\bar{x}$	135	123	139	128	129	120	143	130	134	126
	± S.E. of mean	4·6	5·2	2·8	2·9	6·3	7·0	4·2	4·4	4·2	7·0
	$\bar{d}$	11·8		10·8		9·6		12·8		8·2	
	t	4·9		4·4		2·23		5·0		1·85	
	$2P$	< 0·001		< 0·005		< 0·05		< ·001		< 0·10	
Left atrial pressure (cm H_2O)	$\bar{x}$	11	34	13	35	12	30	15	35	13	35
	± S.E. of mean	3·4	7·9	3·6	4·3	0·6	2·0	1·0	1·6	1·5	1·5
	$\bar{d}$	22·1		21·8		17·9		19·8		21·2	

a positive free water clearance occurred at a time when urine was hypertonic. This apparent contradiction is due to the averaging of values from seventeen tests. In any single test free water clearance was positive only when the urine was hypotonic.

Changes in urine volume during vasopressin infusion. Left atrial distension caused a statistically significant increase ($2P < 0{\cdot}10$) in urine volume during infusions of vasopressin in all but the tests in which vasopressin was infused at 0·4 m-u./kg.min. The increases which occurred during infusion at 0·1, and 1·0 m-u./kg.min were small and although the urine volumes during the control periods in these tests were not significantly different from those during saline infusion the urine volumes during the experimental periods were significantly less ($2P < 0{\cdot}05$) than those observed during saline infusion in the same dogs. There were no statistically significant differences in urine volume between tests with saline infusion and tests during infusion of vasopressin at a rate of 0·025 m-u./kg.min either in the control or experimental periods. Thus the increase in urine flow in response to left atrial distension was not affected by infusion of vasopressin at a rate of 0·025 m-u./kg.min but was significantly reduced by infusion of vasopressin at a rate of 0·1 m-u./kg.min or above.

Changes in urine osmolality during vasopressin infusion. Left atrial distension was associated with a decrease in urine osmolality in all groups of tests (Table 2). The decrease in urine osmolality was not statistically significant in the tests in which the animals received 0·4 or 1·0 m-u./kg.min of vasopressin. During infusion of vasopressin urine osmolality was greater during both the control and experimental periods. However, paired differences between the tests in which saline was infused and those in which vasopressin was infused at a rate of 0·025 m-u./kg.min were not significant. In dogs receiving 0·1 m-u./kg.min vasopressin or more, urine osmolality was significantly greater ($2P < 0{\cdot}05$) than the values during saline infusion in both the control and experimental periods. Urine osmolalities during the control periods with the three highest doses of vasopressin were not significantly different from one another.

In general the low control osmolalities were associated with no infusion of vasopressin and the high osmolalities with high doses of vasopressin although it was also true that in any one dog the osmolality was either generally high (e.g. Fig. 2) or generally low. It would not have been possible to predict from the control osmolality in an individual test the dose of vasopressin being given. Dogs receiving saline infusion had urine osmolalities varying from 108 to 1300 m-osmole/kg and dogs receiving infusion of vasopressin at a rate of 1·0 m-u./kg.min had urine osmolalities from 440 to 1560 m-osmole/kg. Whereas high doses of vasopressin reduced the diuretic response to atrial distension which was then accompanied by only

TABLE 2. Effects of left atrial distension and infusion of vasopressin upon urinary excretion: C control periods, E experimental periods

		Rate of infusion of vasopressin (m-u./kg.min)									
		Saline		0·025		0·1		0·4		1·0	
		C	E	C	E	C	E	C	E	C	E
	n	17		10		9		10		9	
Urine flow (ml./min)	$\bar{x}$	1·25	1·91	1·03	1·39	0·79	0·97	0·92	1·14	0·63	0·71
	± S.E. of mean	0·21	0·29	0·20	0·24	0·19	0·24	0·13	0·19	0·17	0·18
	$\bar{d}$	0·65		0·37		0·18		0·22		0·08	
	t	3·9		3·14		2·18		1·77		2·86	
	$2P$	< 0·005		< 0·01		< 0·10		< 0·20		< 0·02	
Urine osmolality (m-osmole/kg)	$\bar{x}$	616	437	712	536	818	757	835	786	896	866
	± S.E. of mean	87	78	115	93	129	128	100	84	110	108
	$\bar{d}$	177		180		61		48		30	
	t	3·73		3·56		2·65		1·17		1·68	
	$2P$	< 0·005		< 0·01		< 0·05		< 0·30		< 0·20	
Osmolal clearance (ml./min)	$\bar{x}$	1·58	1·81	1·73	2·02	1·73	1·85	2·26	2·72	1·68	1·85
	± S.E. of mean	0·13	0·18	0·24	0·29	0·30	0·34	0·30	0·44	0·39	0·38
	$\bar{d}$	0·23		0·29		0·13		0·46		0·17	
	t	2·44		2·45		1·03		2·22		2·48	
	$2P$	< 0·05		< 0·05		< 0·40		⩾ 0·05		< 0·05	

TABLE 2 (*cont.*)

		Rate of infusion of vasopressin (m-u./kg.min)									
		Saline		0·025		0·1		0·4		1·0	
		C	E	C	E	C	E	C	E	C	E
	n	17		10		9		10		9	
Free water clearance (ml./min)	$\bar{x}$	−0·32	+0·10	−0·70	−0·63	−0·98	−0·88	−1·36	−1·58	−1·05	−1·13
	± S.E. of mean	0·24	0·34	0·24	0·35	0·19	0·25	0·21	0·27	0·24	0·22
	$\bar{d}$	+0·42		+0·08		+0·13		−0·23		−0·08	
	t	2·43		0·49		1·03		2·37		1·72	
	$2P$	< 0·05		< 0·70		< 0·40		< 0·05		< 0·20	
Sodium excretion (m-mole/min)	$\bar{x}$	0·096	0·105	0·107	0·127	0·131	0·151	0·176	0·218	0·138	0·154
	± S.E. of mean	0·026	0·028	0·026	0·028	0·043	0·044	0·030	0·050	0·054	0·055
	$\bar{d}$	0·010		0·021		0·020		0·043		0·016	
	t	0·98		1·6		1·99		1·74		2·16	
	$2P$	< 0·40		< 0·20		< 0·10		< 0·20		< 0·10	
Potassium excretion (m-mole/min)	$\bar{x}$	0·056	0·059	0·063	0·067	0·054	0·055	0·091	0·094	0·056	0·060
	± S.E. of mean	0·007	0·008	0·008	0·008	0·007	0·007	0·012	0·011	0·007	0·007
	$\bar{d}$	0·003		0·004		0·002		0·003		0·004	
	t	1·03		2·31		0·35		0·57		2·24	
	$2P$	< 0·40		< 0·05		< 0·8		< 0·6		< 0·10	

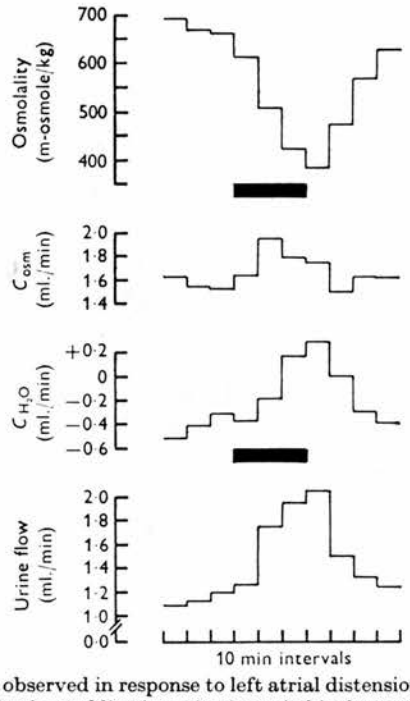

Fig. 1. Changes observed in response to left atrial distension during infusion of saline. Each horizontal line in a 10 min period is the average of seventeen tests in seventeen dogs. The dark box indicates the period of atrial distension. From above downwards urine osmolality, osmolal clearance, free water clearance and urine flow.

small decreases in osmolality a high control osmolality did not of itself prevent a significant diuretic response and decrease in osmolality. In the example shown in Fig. 2 the control osmolality during saline infusion was 1200 m-osmole/kg and left atrial distension caused an increase in urine flow, a large decrease in osmolality and an increase in free water clearance.

Changes in solute excretion during vasopressin infusion. Left atrial distension was associated with a small but statistically significant increase in osmolal clearance in all groups of tests except those in which vasopressin was infused at a rate of 0·1 m-u./kg.min (Table 2). The changes in sodium and potassium excretion which occurred during atrial distension were small and variable. There were seventeen tests out of a total of fifty-five tests of atrial distension in which sodium and potassium excretion decreased while osmolal clearance increased. This fact may account for the observation that osmolal clearance increased while sodium and potassium

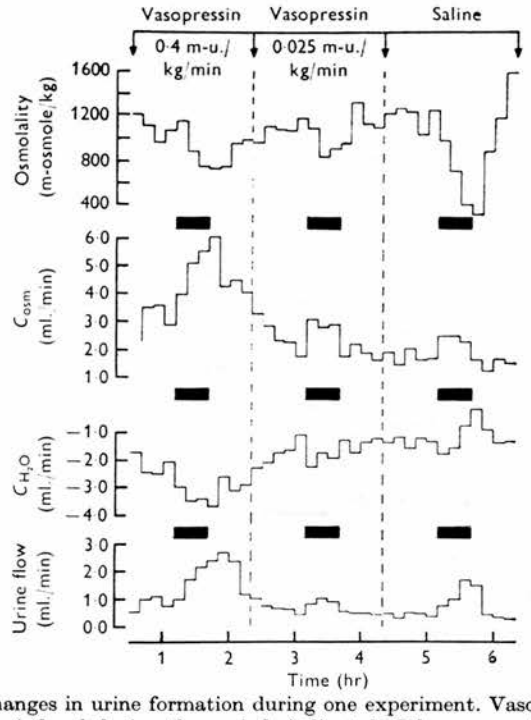

Fig. 2. Changes in urine formation during one experiment. Vasopressin or saline was infused during the periods indicated. Other conventions as in Fig. 1.

excretion remained unaltered. Infusions of vasopressin at rates of 0·025, 0·1, and 1·0 m-u./kg.min had no significant effect on osmolal clearance, sodium excretion or potassium excretion either during the control periods or during the experimental periods. However, these variables were significantly higher during the tests in which vasopressin was infused at a rate of 0·4 m-u./kg.min. This difference appears to have been due to the appearance of an increase in osmolal clearance which coincided with two of the tests in which this dose was used. An example is shown in Fig. 2. Such increases in osmolal clearance occurred most frequently about 1–2 hr after completion of the surgical procedures and have been seen in other groups of experiments (Mason & Ledsome, 1971). The small increase in osmolal clearance associated with left atrial distension appeared to be unaffected by infusion of vasopressin in any of the doses used.

Changes in free water clearance during vasopressin infusion. Left atrial distension caused a large and significant increase in free water clearance

only when the tests were performed during an infusion of saline (Table 2). During infusion of vasopressin at a rate of 0·025 m-u./kg.min the free water clearances during the control and experimental periods were not significantly different from the values during saline infusion but there was no significant increase in free water clearance during left atrial distension. During infusion of vasopressin at a rate of 0·1 m-u./kg.min the free water clearance during the control periods was not significantly less than that during saline infusion but during the experimental periods free water clearance was significantly less ($2P < 0·10$) than that during saline infusion. During infusion of vasopressin at rates of 0·4 and 1·0 m-u./kg.min the free water clearances during the control and experimental periods were significantly less ($2P < 0·05$) than in the experiments in which saline was infused and during atrial distension there was always either no change or a decrease in free water clearance.

Assessment of an effective dose of vasopressin. If the action of vasopressin is to allow increased water reabsorption without affecting osmolal excretion then when a maximum antidiuretic dose of vasopressin is administered changes in free water clearance and osmolal clearance should be dependent upon one another. This relationship is illustrated in Fig. 3. Each point is the average free water clearance and osmolal clearance over each 10 min period from all the experiments during infusion at each dose of vasopressin. Each group consists of ten points, the three 10 min periods before atrial distension, the three 10 min periods during atrial distension and the four 10 min periods after atrial distension. Each group therefore includes all values during the control and experimental periods in that group of tests. There was no correlation between osmolal clearance and free water clearance in the groups of tests during infusion of saline or vasopressin in doses of 0·025 m-u./kg.min and 0·1 m-u./kg.min. During infusion of vasopressin at rates of 0·4 m-u./kg.min and 1·0 m-u./kg.min there was a highly significant correlation ($2P < 0·001$) between osmolal clearance and free water clearance. The regression lines for the latter two groups of points were not significantly different. The combined regression line is shown in Fig. 3.

Effects of atrial distension on plasma composition. During the control periods the average plasma osmolality in all nineteen dogs was $289 \pm 1·0$ (S.E. of mean) m-osmole/kg, plasma sodium concentration was 140 m-equiv/l. and plasma potassium concentration was 4 m-equiv./l. There were no significant changes in plasma osmolality or sodium or potassium concentrations during left atrial distension.

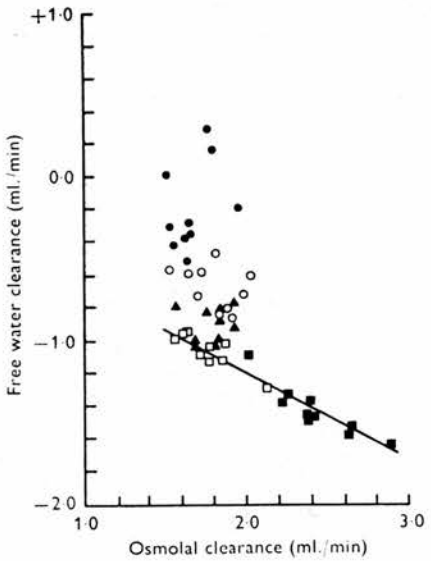

Fig. 3. Average free water clearance and osmolal excretion in all experiments. Each point is the average value for a 10-min urine collection period during infusion of saline or vasopressin. ● saline, ○ vasopressin 0·025 m-u./kg.min, ▲ vasopressin 0·1 m-u./kg.min, ■ vasopressin 0·4 m-u./kg.min, □ vasopressin 1·0 m-u/kg.min. Each group consists of ten points, the three 10 min periods before atrial distension, the three 10 min periods during atrial distension and four 10 min periods after atrial distension. The line represents the calculated regression line for the points during infusion of vasopressin at 0·4 and 1·0 m-u./kg.min. The equation for this line is $-0{\cdot}14 = (-1{\cdot}25) - (-0{\cdot}54)(2{\cdot}07)$, $a = \bar{y} - b\bar{x}$.

DISCUSSION

The cardiovascular changes induced by left atrial distension were similar to those which have been reported previously (Ledsome *et al.* 1961; Arndt *et al.* 1963). These changes were not altered by infusion of vasopressin in any of the doses used (Table 1). In both groups of experiments the average heart rates decreased and mean arterial pressures increased with increasing doses of vasopressin but these effects were not statistically significant. Any differences in the urinary responses during infusion of vasopressin are therefore unlikely to be due to vascular effects of the vasopressin or to altered cardiovascular responses to atrial distension.

The diuretic responses to left atrial distension in those tests which were carried out during saline infusion were qualitatively similar to those previously reported in other series (Ledsome *et al.* 1961; Arndt *et al.* 1963).

The diuretic responses were quantitatively somewhat smaller than previously reported, but in earlier studies (Henry *et al.* 1956; Ledsome *et al.* 1961) tests of atrial distension were carried out only after urine flow had remained steady for a period of time (usually 40 min), whereas in the present series a strict protocol was maintained regardless of changing urine flow. Doses of vasopressin were given in different order and it is unlikely that the average changes attributed to atrial distension were the result of spontaneous variations or due merely to changing the rate of vasopressin infusion. In another series of experiments (Mason & Ledsome, 1971) in which infusions of vasopressin were given, but atrial distension was not performed, no such rapid variations in urine flow were observed.

The results observed during infusion of vasopressin at a rate of 0·025 m-u./kg.min confirm the observations of Ledsome *et al.* (1961) that infusion of vasopressin at this dose does not prevent the increase in urine volume or the decrease in osmolality which occurs during left atrial distension. However, there was no significant increase in free water clearance in response to atrial distension during infusion of vasopressin at 0·025 m-u./kg.min although increases in free water clearance were observed in individual experiments. A similar effect was described by Lydtin & Hamilton (1964) in unanaesthetized hydrated dogs. Higher doses of vasopressin (0·4 m-u./kg.min and above) completely prevented the increase in free water clearance, prevented a significant decrease in osmolality and reduced the increase in urine volume in response to atrial distension. The small increase in osmolal clearance associated with left atrial distension appeared to be unaffected by vasopressin infusion.

It was suggested previously (Ledsome *et al.* 1961) that because a diuretic response to left atrial distension occurred during infusion of vasopressin in doses of 0·025 m-u./kg.min and 0·1 m-u./kg.min the diuresis could not be due to decreased release of antidiuretic hormone from the neurohypophysis. This conclusion was based on the data of Verney (1947) and Shannon (1942) who demonstrated in conscious dogs undergoing water diuresis maximum decreases in urine flow with doses of vasopressin of 0·01 m-u./kg.min. Also assay of the vasopressin used in the previous experiments (Ledsome *et al.* 1961) had shown that a dose of 0·009 m-u./kg.min was adequate to prevent the appearance of a water diuresis in a conscious dog. However, it is apparent that in anaesthetized dogs the effectiveness of vasopressin in concentrating the urine is dependent upon the osmolal clearance (Orloff, Wagner & Davidson, 1957) and upon the state of hydration of the animal (Perlmutt, 1962). Recent experiments (Mason & Ledsome, 1971) have demonstrated that in dogs anaesthetized with chloralose and hydrated in a similar fashion to the present series a transient diuresis may be produced when infusion of vasopressin at a high rate (0·4 m-u./

kg.min) is reduced to a lower but usually antidiuretic dose (0·04 m-u./kg.min). The results of Mason & Ledsome (1971) and the present results both indicate that infusion of vasopressin at a rate of 0·025 m-u./kg.min does not constitute a maximally effective antidiuretic dose under the conditions of hydration and anaesthesia described. The results plotted in Fig. 3 suggest that infusion of vasopressin at a rate of 0·4 or 1·0 m-u./kg.min was required to prevent changes in free water clearance from occurring independently of changes in osmolal clearance and only these doses represent maximally effective doses of vasopressin. The results allow the conclusion that a part of the diuretic response to left atrial distension could depend upon a decrease in the concentration of antidiuretic hormone in the circulating blood.

Doubts as to the physiological significance of such a mechanism have been expressed (Lydtin & Hamilton, 1964). Also Ledsome *et al.* (1961) suggested that if the neurohypophysis was involved the effect would be so far removed from the normal function of this structure as to have no relevance in the study of the normal control of urinary volume. However, some of the more puzzling features of the diuretic response to left atrial distension have now been explained. It has become apparent that in the anaesthetized dog the kidneys are capable of producing a transient dilution of the urine in response to changes in vasopressin infusion which might be considered outside the physiological range in the conscious dog (Mason & Ledsome, 1971). Also relatively large changes in antidiuretic activity in the circulating blood have been demonstrated during left atrial distension (Shu'ayb *et al.* 1965; Johnson *et al.* 1969). Finally, the increase in free water clearance associated with left atrial distension is prevented only when infusions of vasopressin in a high dose range are used. It seems reasonable to suppose that in the conscious dog a decrease in antidiuretic activity could occur during atrial distension possibly at a lower range of concentration leading to a greater increase in free water clearance which would be inhibited by smaller doses of vasopressin. Lydtin & Hamilton (1964) provide some support for this speculation.

When a diuretic response is induced in an anaesthetized dog by decreasing the rate of an infusion of vasopressin there is an increase in free water clearance and no change or a decrease in osmolal clearance (Mason & Ledsome, 1971). Atrial distension has been shown to be accompanied by both an increase in free water clearance and an increase in osmolal clearance. The increase in osmolal clearance in response to atrial distension was unaffected by large doses of vasopressin and is unlikely to be caused by a decrease of antidiuretic activity in the blood. Two explanations are possible; firstly there may be two agents affecting the kidney to produce a diuretic response, a decrease in circulating levels of antidiuretic hormone

and an unknown agent, possibly a haemodynamic change (Arndt *et al.* 1963) producing an increased osmolal clearance; secondly, one unidentified agent may act upon the kidney, the characteristics of the diuretic response being determined by the concentration of circulating antidiuretic hormone. The first explanation now appears to be the more likely.

The authors are indebted to Mrs G. Bennion for her assistance and are grateful for support from the Medical Research Council of Canada.

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With 5 text-figures
Printed in Great Britain

THE EFFECTS OF DECEREBRATION ON THE REFLEX RESPONSE TO PULMONARY VEIN DISTENSION

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(*Received 6 June 1972*)

SUMMARY

1. A method is described for the decerebration of dogs using high frequency coagulation. Animals made decerebrate by this method showed a slowing of the heart rate and a decrease in arterial pressure.

2. Distension of the pulmonary vein–left atrial junctions by the inflation of small balloons caused an increase in heart rate in intact and decerebrate dogs. The magnitude of the response was not significantly different in the two states.

3. The increase in heart rate caused by pulmonary vein distension was shown to be a reflex. It was significantly reduced by injection of propranolol both before and after decerebration. Cervical vagotomy always prevented any response.

4. The similarity of the responses before and after decerebration suggests that structures rostral to the superior colliculus are not required for the appearance of the full reflex effect.

5. The magnitude of the response remaining after administration of propranolol raised the question of the efficacy of propranolol as a β-blocking agent in this experimental situation, or alternatively suggests the possibility of the existence of an efferent vagal component to the reflex response.

INTRODUCTION

An increase in heart rate in response to distension of the pulmonary vein-left atrial junctions by small balloons has been previously described (Ledsome & Linden, 1964). This response is a reflex with its afferent pathway in the vagus nerves and with its efferent pathway thought to be solely in the cardiac sympathetic nerves (Ledsome & Linden, 1964, 1967; Furnival, Linden & Snow, 1971). The reflex is remarkable in that the increase in heart rate has been shown to occur with little or no change in

vascular resistance in the hind limb (Carswell, Hainsworth & Ledsome, 1970) or in cardiac contractility (Furnival *et al.* 1971). It has also been shown that altering the perfusion pressure in the carotid arteries does not affect the response to pulmonary vein distension (Carswell *et al.* 1970). Thus it seems likely that the reflex response to pulmonary vein distension does not interact with arterial baroreceptor reflexes and that the central nervous connexions of these reflexes might be different. The experiments described were designed to study the effects of decerebration on the reflex response to pulmonary vein distension and represent the first stage in an attempt to localize the region of central nervous control.

METHODS

One-half hour before the anaesthetic was administered mongrel dogs of either sex, 8–16 kg, were given morphine sulphate 0·5 mg/kg s.c. Under local anaesthesia (Winthrop Laboratories: carbocaine, 1%), a cannula was inserted in the saphenous vein and α chloralose (British Drug Houses: 1% (w/v) solution in 0·9% sodium chloride) infused in a dose of 10 ml./kg body weight. The level of anaesthesia was maintained throughout the experiment by the addition of approximately 10% of the original dose every half hour both before and after decerebration. The dog's oesophageal temperature was kept constant at 37° C ($\pm$ 2) by a heated table.

The right femoral artery was cannulated with a 6 in. length of Teflon tubing (1 mm bore), and femoral arterial pressure measured using a strain gauge manometer (Statham, P23Gb). After amplification by a DC amplifier (Honeywell Accudata 113) pressure was recorded on a direct writing ultra violet light recorder (Honeywell, Model 1508). The frequency response of the system measuring arterial pressure, tested by the method of Hansen (1949), was flat ($\pm$ 5%) to better than 35 Hz. Mean pressure was obtained electrically. Samples of arterial blood were taken at intervals throughout the experiment and P_{CO_2}, P_{O_2} and pH measured using appropriate electrodes and an Instrumentation Laboratories blood gas analysing system. Additions of sodium bicarbonate (1 M) or adjustments in the respiratory pump stroke volume were used to keep pH and P_{CO_2} within the normal ranges of 7·3–7·4 pH units, and 35–40 mm Hg, respectively.

A standard 2-lead e.c.g. was attached to the chest and after pre-amplification (Grass Instruments; P15) was displayed simultaneously on the ultra-violet recorder and dual beam oscilloscope (Tektronix type, Rm 565). Heart rate was also recorded using a cardiotachometer (Honeywell) triggered by the R wave of the e.c.g. All heart rates used in the experimental results were counted from the e.c.g. record over periods of at least 0·5 min.

The animal was then turned so that the left side was exposed and the chest opened at the 5th intercostal space. A Harvard respirator was attached to the tracheal cannula with a stroke volume of approximately 50 ml./3 kg body weight at a rate of 18 breaths/min. One litre per minute of oxygen was added to the inspired air to ensure adequate oxygenation. Once the chest was opened a resistance of 3 cm H_2O was added to the expiratory outlet. Dextran (Travenol, Baxter Laboratories) was infused to approximate 10% of the dog's estimated total blood volume (8% of body weight).

After deflecting the left lung so as to expose the pulmonary veins, small balloons were inserted in the veins after the manner described by Ledsome & Linden (1964). The left lung root was then tied off completely with stout cord.

Decerebration was accomplished by means of a high frequency coagulation system modified from that described by Koller & Jenny (1969) for the decerebration of small mammals. Electrodes were made of 23-gauge stainless-steel tubing cut into 17 cm lengths and firmly fixed with epoxy cement into an electrode holder to form a fork-like apparatus with nine tines, each 2 mm apart. When completed the electrodes varied in length from 64 mm at the extremities to 67 mm at the mid point, being tapered at both sides so as to fit the base of the skull. To insulate the electrodes they were slowly lowered into a beaker of thinned Insul-X (Insul-X Products, Yonkers, N.Y.), then baked at 70° C for 1 hr. This process was repeated three times, or until a thin even coat of insulation covered the electrodes. Checks for breaks in the coating were made using a resistance meter. At the tip of each electrode 2–3 mm were then scraped bare to allow the passing of high frequency current between the tips of two adjacent electrodes.

To perform decerebration using this system the dog's head was firmly fixed in a stereotaxic frame (La Precision Cinematique, Paris). The ear bars were at 40 mm posterior to zero on the stereotaxic frame and the head was fixed so that the eyes were always in the same horizontal plane as the ear bars. This procedure provided standardization of position despite wide variations in head size and shape. The skin over the skull was cut in the mid line and the superficial muscles of the head deflected laterally. Trephination was accomplished using a small hand drill. A rectangular hole approximately 10 × 40 mm was prepared, lying across the mid line, and bone wax was used to seal the edges of the rectangle and prevent bleeding. The position of the hole was determined by placing the electrode holder in the same transverse plane as the ear bars; this placed the electrodes 10 mm rostral to the ear bars because of the design of the electrode holder. Experience had shown that lowering the electrodes at these co-ordinates, perpendicular to the plane of the frame, effectively severed the brain stem at the mid-collicular level. Before lowering the electrodes, the dura was opened with fine scissors on either side of the sagittal sinus. Care had to be taken when entering the brain that the centre electrodes separated over the sinus without undue splaying. The electrodes were then lowered until the resistance of the bone at the base of the skull was felt, usually about 40 mm from the surface. The animal was made decerebrate by passing a current of approximately 50–100 mA for 15 sec between the tips of each pair of adjacent electrodes using a Wyss coagulator (J. Monti, Geneva). Coagulation was performed at ten successive 2 mm steps up from the base of the skull.

Although 'decerebrate rigidity' was never observed in animals prepared in this manner, functional signs of a complete decerebration were extension of the limbs, reflex muscular contraction, bladder incontinence, and frequently a reduction in both heart rate and blood pressure. Anatomical evidence of decerebration was observed at the completion of each experiment by examining the brain stem after exsanguination. Careful removal of the cerebral hemispheres by severing the cerebral peduncles revealed a region of destroyed tissue at the mid-collicular level. Usually the forebrain fell away in a clean line from the brain stem, showing an area softened and occasionally blackened. If any tissue had not been coagulated it remained firm and white and no amount of probing could make it resemble the destroyed tissue. Small strands of such uncoagulated tissue were seen in only two dogs. Further evidence of the extent and effectiveness of the lesion produced was demonstrated by preparing a sagittal section of the head of a dog made decerebrate by this system. The section was obtained by bleeding the animal, removing the head, and immediately freezing it. One week later the head was sectioned with a bandsaw, and revealed an area of softening through the brain stem, about 5 mm across, immediately caudal to the level of the superior colliculus.

When β-receptor blockade was desired, propranolol, 0·5 mg/kg (Ayerst Laboratories; Ay-64043) was administered through the saphenous vein cannula followed by a 5 ml. wash of saline. Satisfactory β blockade was judged on the basis of the ability of the injected propranolol to block 90 % or more of the maximum increase in heart rate caused by rapid intravenous injection of isoprenaline 0·5 μg/kg (K & K Laboratories Inc.; isoprenaline salt sulphate).

Experimental protocol. After general surgery and opening of the skull and dura had been completed the animal was allowed to recover for at least 10 min, or until a steady state of blood pressure and heart rate had been achieved, before beginning the period of experimentation. Testing for the presence of the pulmonary vein–left atrial reflex was always done in the same manner. Following a control period, the pulmonary vein balloons were inflated by injection of 0·5 (8 to 11 kg dogs) or 1 ml. (12 to 18 kg dogs) of saline for 2 min, after which a record was made for 1 min during the period of inflation and the balloons then deflated. After a 2 min recovery period, another one minute record was taken, and changes in heart rate and blood pressure calculated from mean values before and after balloon inflation. When three such trials had been completed, the animal was made decerebrate using the high frequency electrode system, and, after equilibration, the reflex testing procedures repeated. The significance of the changes in heart rate and blood pressure was tested using a t test for paired data with a Student's range.

RESULTS

Effects of decerebration. Because it is of importance in assessing the relative magnitude of any changes in heart rate or blood pressure, the effects of decerebration on control values of these parameters will be considered before examining in detail the influence of decerebration on the response to pulmonary vein distension. In thirty-six dogs made decerebrate by the electrocoagulation method, heart rate decreased in twenty-seven animals and blood pressure decreased in twenty-eight; mean heart rate fell from 142 beats/min (S.E. of mean $\pm$6·6, range 84–228) to 120 beats/min (S.E. of mean $\pm$8·4, range 48–240) following decerebration. Blood pressure followed a similar pattern, decreasing from control values of 119·5 mm Hg (S.E. of mean $\pm$3·2, range 80–152 mm Hg) to 106·3 (S.E. of mean $\pm$2·6, range 72–156). The changes were statistically significant ($2P < 0{\cdot}005$), occurred immediately upon section of the brain stem at the mid-collicular level, and were in general substained during the 4–6 hr of experimentation after 'recovery' from decerebration. Even in six dogs which had been treated before decerebration with the β blocking agent propranolol, heart rate decreased from an average of 112 beats/min before decerebration to 93 beats/min after decerebration, indicating that much of the change in heart rate was due to a change in vagal tone.

Effects of inflating balloons at the pulmonary vein–left atrial junctions. Inflation of balloons in the pulmonary veins in animals with the dura opened but before decerebration caused a significant increase in heart rate without significant change in femoral arterial mean pressure. In a total of

109 trials in thirty-two dogs the average increase in heart rate was 18 beats/min (S.E. of mean ± 1·5) and the average change in arterial mean pressure was − 1·4 mm Hg (S.E. of mean ± 0·4). The increase in heart rate developed gradually over 10 sec–2 min and was similar in magnitude to that reported by Ledsome & Linden (1964).

In a total of eighteen dogs the effects of inflating balloons at the pulmonary vein-left atrial junctions were tested both before and after decerebration. In these eighteen dogs before decerebration, fifty-four trials of pulmonary vein distension caused an average increase in heart rate of 21 beats/min (S.E. of mean ± 2·4) and a change in arterial mean pressure of

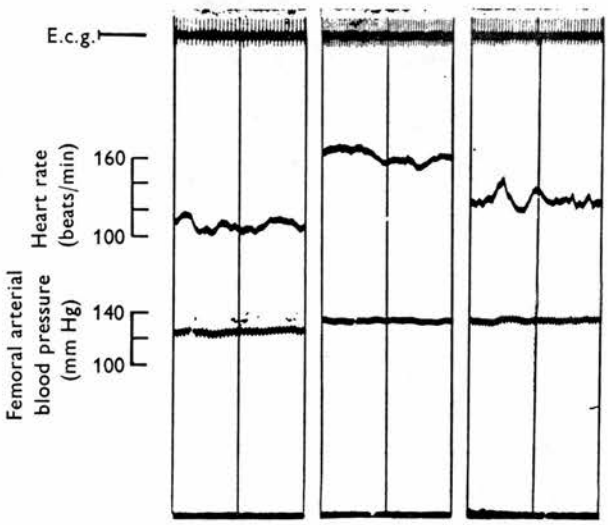

Fig. 1. A record of the response to distension of the pulmonary vein–left atrial junction in a dog anaesthetized with chloralose. In sequence from left to right are shown sections of the record during the control period, during inflation of the pulmonary balloons, and 2 min after deflation. Each vertical time line represents 10 sec.

− 2·2 mm Hg (S.E. of mean ± 0·6). After decerebration fifty-four trials of pulmonary vein distension caused an increase in heart rate of 21 beats/min (S.E. of mean ± 1·9) and a change in arterial mean pressure of 1·3 mm Hg (S.E. of mean ± 0·8). When compared statistically there was no significant difference between the changes in heart rate and blood pressure before and after decerebration in the same dogs. An example of the record in a representative experiment is shown in Figs. 1 and 2.

Reflex nature of the response to pulmonary vein distension in the decerebrate dog. It was reported by Ledsome & Linden (1964) that the response to pulmonary vein distension was of reflex origin, the afferent pathway being

in the vagus nerves, and the efferent pathway in the cardiac sympathetic nerves. To determine whether the reflex response had been altered in any way by the removal of higher centres, the effect of the sympathetic β blocking agent propranolol was tested and cervical vagotomy subsequently performed.

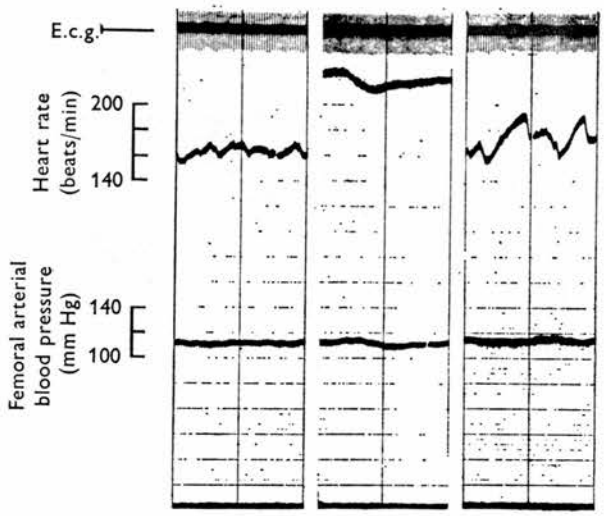

Fig. 2. A record of the response to distension of the pulmonary vein–left atrial junction in a decerebrate dog anaesthetized with chloralose. Conventions as in Fig. 1. This record was taken from the same dog as in Fig. 1. In this animal heart rate was increased after decerebration.

Eleven decerebrate dogs were given propranolol, 0·5 mg/kg, a dose sufficient to block 90% or more of the increase in heart rate caused in response to β-receptor stimulation by isoprenaline, 0·5 μg/kg, injected I.V. The β blockade was maintained throughout the period of reflex testing by giving additional doses of propranolol (0·5 mg/kg) whenever injection of isoprenaline elicited an increased response. In thirty-two trials of pulmonary balloon inflation in these eleven dogs, the reflex increase in heart rate was significantly reduced ($2P < 0{\cdot}01$) from 15 beats/min (S.E. of mean $\pm$ 1·3, range 3–76) before administration of propranolol to 8 beats/min (S.E. of mean $\pm$ 1·1, range 0–25), after β blockade. Blood pressure changes were small, and not significantly different. The changes in heart rate occurred despite the fact that the administration of propranolol decreased the response to β-receptor stimulation with isoprenaline (0·5 μg/kg) from an increase in heart rate of 90 beats/min (S.E. of mean $\pm$ 11·7, range 24–186), and a decrease in blood pressure of -41 mm Hg (S.E. of mean, $\pm$ 3·9, range -10–(-72)) to 2 beats/min (S.E. of mean $\pm$ 1·3,

range 16–(−6)) and −1·3 mm Hg (S.E. of mean ±1·0, range 6–(−16)). As the increase in heart rate remaining in response to pulmonary vein distension was greater than expected from the results of Ledsome & Linden (1967) and Furnival *et al.* (1971), the possibility existed that decerebration had in some manner altered the reflex response to pulmonary vein

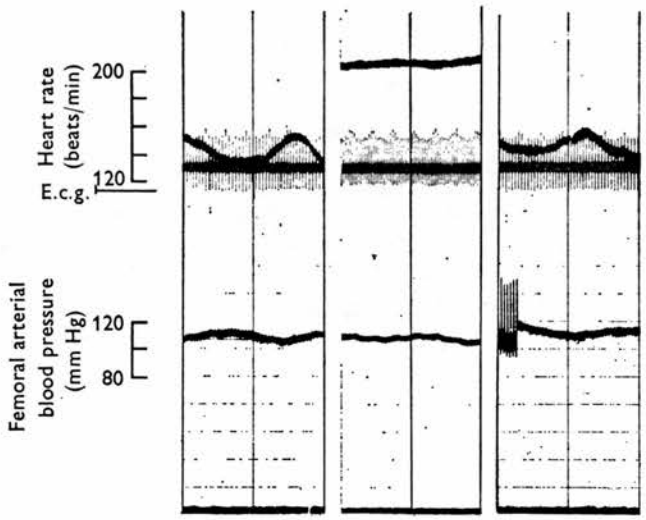

Fig. 3. Response to pulmonary vein–left atrial distension in an intact dog anaesthetized with chloralose. Conventions as in Fig. 1.

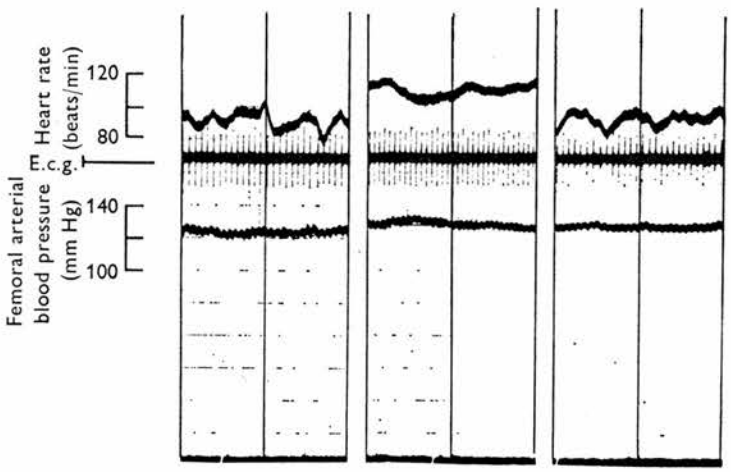

Fig. 4. Response to pulmonary vein–left atrial distension in an intact dog following β blockade with propranolol (0·5 mg/kg). Conventions as in Fig. 1. This record was taken from the same dog as that in Fig. 3.

distension. In order to test this possibility a series of experiments was conducted in which propranolol was administered prior to decerebration, and the β blockade maintained following decerebration. In six dogs, although the response was significantly reduced both before and after decerebration, it was not abolished. In the eighteen trials in the intact animals, heart rate increases to balloon inflation were reduced by β blockade from control values of 23 beats/min (S.E. of mean $\pm 3{\cdot}5$, range 7–62) to 6 beats/min (S.E. of mean $\pm 1{\cdot}3$, range 0–15·5). In the twenty trials in the same animals

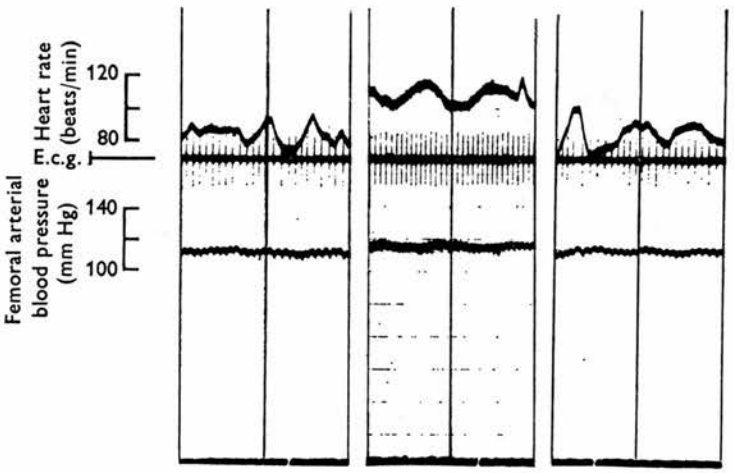

Fig. 5. Response to pulmonary vein–left atrial distension in a decerebrate dog following β blockade with propranolol (0·5 mg/kg). This record was taken from the same dog as in Figs. 3 and 4. Conventions as in Fig 1. In this dog both heart rate and blood pressure were decreased after decerebration.

when decerebrate, the response was 8 beats/min (S.E. of mean $\pm 1{\cdot}2$, range 0–21), which was not significantly different from the intact, β blocked values. Propranolol reduced the control heart rates by similar amounts when given either before or after decerebration. In the six intact animals heart rate was reduced from 136 beats/min to 120 beats/min. In the eleven decerebrate animals the change was from 117 to 106 beats/min. It may be noted from the ranges of the responses given that propranolol did completely prevent the response in some animals but that in others a significant response remained. The mean percentage of the control response remaining after propranolol in the six intact dogs was 26 %, increasing to 35 % after decerebration. In the eleven dogs given propranolol only after decerebration, 53 % of the control response remained. However, in both groups of animals anywhere from 0 to 100 % of the full increase in heart rate occurring at pulmonary vein distension remained after the admini-

stration of propranolol. Results from a typical experiment are shown in Figs. 3, 4 and 5. In all cases the remaining response was totally abolished by cervical vagotomy.

DISCUSSION

These investigations, though they cannot with precision localize the central synapses for the reflex increases in heart rate caused by pulmonary vein distension, can indicate the extent of central control necessary for implementation of the full reflex response. Decerebration at the mid-collicular plane appeared to cause an increase in cardiac vagal tone as evidenced by the bradycardia which occurred in the presence of propranolol, and possibly also a decrease in sympathetic vasoconstrictor tone as evidenced by the fall in arterial pressure. These cardiovascular changes are in contrast to the increase in arterial pressure reported in cats, rabbits and guinea-pigs (Koller & Jenny, 1969). This may represent a species difference or an effect of the chloralose anaesthetic used in the present experiments. However, despite the alteration of the cardiovascular background, decerebration did not significantly alter the magnitude of the response to pulmonary vein distension, or the magnitude of the portion of the response remaining after administration of propranolol. Thus it seems unlikely that structures rostral to the superior colliculus, such as the hypothalamus, are required for the appearance of the full reflex effect. Consequently, it must be concluded that the region of central nervous system control is located either in the brain stem, or at the spinal level. Although the spinal cord, in the absence of the brain, appears to be capable of increasingly complex circulatory adjustments (Katunsky & Khayutin, 1968; Khayutin & Lukoshkova, 1970), we have no specific evidence relating the left atrial reflex to spinal mechanisms. However, before dismissal of this possibility further experiments would be required.

On the other hand, there are several pieces of evidence which might link the left atrial reflex to the brain stem, and more specifically, to the medulla. The presence of the classical cardiovascular reflex centres in the medulla make it likely that connexions of the left atrial reflex might terminate in the same region. Additional evidence may exist in the studies by Calaresu & Henry (1971) of the effects of stimulation of the 'para-hypoglossal' area (PHA) in the medulla of the cat. This region of the floor of the fourth ventricle includes the hypoglossal interfascicular nuclei, the medial longitudinal fasciculus (MLF), and the paramedian reticular nucleus (PMRN). Careful nerve degeneration studies by Brodal, Anders & Gogstad (1957) have demonstrated extensive afferents to the PMRN from many regions, and direct projections of carotid sinus nerve fibres to the PMRN have recently been revealed (Homma, Miura & Reis, 1970). Electrical

stimulation of the PHA in the cat (Calaresu & Henry, 1971) caused short latency (1–5 sec) increases in heart rate and blood pressure either together or separately. These increases were not affected by decerebration, and could be significantly decreased by infusions of propranolol, or abolished by cervical vagotomy. Increases in potentials were recorded peripherally in the cardiac sympathetic nerves. Although an increase in pressure is not a feature of the response to pulmonary vein distension, and there is no evidence that this region is the site of central synapses for the tachycardia observed on distension of the pulmonary veins, the similarities in the response are striking, and it would be interesting to test the response to left atrial distension after discrete lesions of the PHA, specifically the PMRN.

A second interesting point which deserves notice in the present investigations is the fact that propranolol did not completely abolish the response to pulmonary balloon inflation. Examination of data on this point in papers published previously (Ledsome & Linden, 1967, 1968; Carswell *et al.* 1970; Ledsome & Hainsworth, 1970) shows that in ten tests in ten dogs after administration of propranolol the average increase in heart rate on pulmonary vein distension was 4·8 beats/min, and in addition Furnival *et al.* (1971) claim that in three dogs the response was abolished although no values were given. In the present experiments despite the fact that propranolol decreased the response to β-receptor stimulation with isoprenaline by more than 90 % in all tests, anywhere from 0–100 % of the full response to pulmonary vein distension remained in individual dogs. This may raise some doubt as to the efficacy of the β blocking action of the dose of propranolol used and of our index of the degree of β blockade, viz. isoprenaline. Some allowance of course must be made for the nature of a competitive blocking agent and absolute block might not be expected. Still the range of percentages of control response to pulmonary vein distension remaining after propranolol indicates that a more precise knowledge of the comparative ability of propranolol to block β-receptor excitation by isoprenaline, cardiac sympathetic nerve stimulation or infusions of noradrenaline would be of value. Concomitantly one should consider the alternative possibility of a contribution to the reflex response to pulmonary vein distension by an efferent vagal component.

This work was supported by the Medical Research Council of Canada and the British Columbia Heart Foundation.

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Quarterly Journal of Experimental Physiology (1973) 58 219–227

Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY,
VOL. LVIII, 1973

THE TIME COURSE OF THE DIURETIC RESPONSE TO LEFT ATRIAL DISTENSION.

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(*Received for publication* 19*th September* 1972)

Distension of the left atrium for a 30 min period causes an increase in urine flow, osmolal clearance and free water clearance. The present experiments examine in detail the changes in urinary excretion during a 90 min distension of the left atrium. The experiments demonstrate that in anaesthetized dogs when distension of the left atrium is continued for a 90 min period the increases in urine flow, solute excretion, and free water clearance are transient. Urine flow, free water clearance, and urine osmolality returned completely to control values only after release of left atrial distension. In contrast, solute excretion returned to control values 40 min after the start of left atrial distension and was independent of any concurrent changes in the dilution of the urine. The results support the hypothesis that the diuretic response to left atrial distension is dependent upon two mechanisms: a decrease in the rate of release of antidiuretic hormone from the neurohypophysis, which may be prolonged; and a haemodynamic mechanism which under the conditions of the present experiments appears to be short lived.

Distension of the left atrium for a period of 30 min causes an increase in urine flow, solute excretion and free water clearance in anaesthetized [Henry, Gauer and Reeves, 1956; Ledsome, Linden and O'Connor, 1961; Arndt, Reineck and Gauer, 1963; Ledsome and Mason, 1972] and unanaesthetized dogs [Lydtin and Hamilton, 1964]. When distension of the left atrium was continued for a time period longer than 30 min the increase in urine flow was transient [Henry *et al.*, 1956; Ledsome *et al.*, 1961; Lydtin and Hamilton, 1964]. However, details of the time course of the transient increase in urine flow and the changes in solute excretion during prolonged left atrial distension have not been previously examined in systematic fashion.

The present investigation was carried out to determine the effects of a 90 min distension of the left atrium and thus provide information regarding the characteristics of the diuretic response to prolonged distension of the left atrium.

METHODS

Dogs of 12–19 kg were given a subcutaneous injection of morphine sulphate (0·5 mg/kg). One hour later under local anaesthesia (mepivacaine hydrochloride, 1%) a catheter was inserted through a saphenous vein into the inferior vena cava and each animal was anaesthetized by an intravenous infusion of 0·1 g/kg of chloralose (British Drug Houses), dissolved to make a solution of 1 g of chloralose in 100 ml. of sodium chloride solution (0·6 g/100 ml.). Subsequently during the experimental procedures a steady state of light anaesthesia and fluid input was maintained by the constant infusion of a 0·5% chloralose solution (0·5 g of chloralose in 100 ml. of 0·6% sodium chloride solution) delivered by a motor driven syringe pump (Harvard Apparatus Co. Inc.) at a rate of approximately 1·0 ml./min. In the 5 largest dogs (18–19 kg) the rate of infusion was increased to 1·80 ml./min.

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As soon as possible after the induction of anaesthesia artificial respiration was started with a mixture of 40% oxygen in air, supplied from a respiration pump (Harvard Apparatus Co., model 614) the rate (about 18/min) and stroke (about 50 ml./3 kg body wt.) of which were adjusted to equal approximately those of the animal's spontaneous respiration. When the chest was opened a resistance to expiration equivalent to 3 cm H_2O was provided by an exhalation valve (Ohio Chemical City). At intervals during the procedures samples of arterial blood were taken and pH, P_{CO_2} and P_{O_2} measured using appropriate electrodes (Instrumentation Laboratories Inc. City, No. 113–51). Adjustments were made to the respiratory pump or small infusions (10–20 m-equiv) of sodium bicarbonate solution (1 M) were given to maintain $PaCO_2$ between 35 and 40 mm Hg and pH within the range 7·3–7·4; no adjustments were made during the control or experimental periods.

Each ureter was catheterized through a flank incision and urine volume was measured every 10 min. The left side of the chest was opened in the fifth intercostal space and a balloon placed in the left atrium as described previously [Ledsome *et al.*, 1961]. Eight of the dogs to be included in this report, in preparation for another experiment, also had a small region (1 cm^2) of their cerebral cortex exposed.

Femoral arterial pressure was recorded through an 8 cm length of teflon tubing (1 mm bore), and left atrial pressure through a 15 cm length of teflon tubing (1 mm bore). Zero pressure was determined *post-mortem* as the level of the tip of the cannula free in air. To each cannula was attached a Statham strain gauge (Model P_{23} Gb), and after amplification by means of a d.c. amplifier (Honeywell, Accudata 113) the pressure was recorded on an ultraviolet light recorder (Honeywell, Visicorder 1508). The frequency response of the system recording femoral arterial pressure, obtained by the method of Hansen (1949), was flat (±5%) to better than 35 Hz. Mean pressures were obtained electrically. Values were recorded every 10 min at the midpoint of the urine collection period.

During the surgical procedures, about 2 hr, the animals received a slow infusion of 100 ml. dextran (6% dextran 75 in 0·9% sodium chloride, Travenol Laboratories Inc.) for each 13 kg body weight (approximately 10% of their blood volume). The electrocardiogram was recorded from leads on the forelegs and chest wall; heart rates were counted from the electrocardiogram over periods of at least 30 sec. Oesophageal temperature was maintained at 37°C±1·5°C using a heating pad and temperature controller (Yellow Springs Instrument Co.).

Samples of arterial blood (3 ml.) were taken during the control and experimental periods into syringes moistened with heparin (500 u/ml., Nutritional Biochemicals Corp.) and the blood was centrifuged immediately; the volume removed was replaced with dextran. Urine and plasma were analyzed for sodium and potassium using a flame photometer (Instrumentation Laboratories Inc., model 143). The average difference between duplicate estimations was 1·0 m-equiv/l. Urine and plasma osmolality were measured by freezing point depression (Osmette, Precision Systems). The average difference between duplicate estimations was 1·0 m-osmole/kg.

Experimental Protocol

Tests of atrial distension were carried out on seventeen dogs. Because the experiments were intended to examine the characteristics of a previously established response rather than to prove the existence of the response, a selection was made to include only those experiments in which the urine flow during the control period was greater than 0·5 ml./min. This selection minimized the effects of dead space in the urine collection system (about 1 ml. in each ureteral cannula) and small spontaneous variations in urine flow. Four dogs were rejected on this basis before commencing the experiment. The results report the effects of 18 tests of atrial distension in thirteen dogs.

Urine collection began after the surgical procedures were completed. One hour

later the left atrial balloon was distended with enough saline (about 1 ml./kg) to increase left atrial pressure by about 20 cm H_2O and distension was maintained for 90 min. Urine collection was continued for 40 min after release of the distension. In five of the dogs two tests of atrial distension were performed. In these dogs the second distension was performed 100 min after release of the first distension.

The control values were taken to be the average of the values in the three 10 min periods preceding atrial distension and the three 10 min periods following release of the distension. The experimental value was the average of the nine 10 min periods during atrial distension. Values for the control and experimental periods were compared using the Student's *t*-test for paired data.

Results

Cardiovascular effects

Distension of a balloon in the left atrium raised left atrial pressure from a control value of 9 cm H_2O (S.E. of mean$\pm$1·1) to an experimental value of 28·0$\pm$1·5 cm H_2O. The heart rate increased ($P < 0{\cdot}001$) from a control value of 129$\pm$9·5 beats/min to an experimental value of 177$\pm$8·8 beats/min and mean arterial pressure decreased from a control ($P < 0{\cdot}025$) value of 128$\pm$5·5 mm Hg to an experimental value of 122$\pm$6 mm Hg. These changes in the cardiovascular variables occurred immediately after atrial distension and generally remained unaltered during the 90 min period of atrial distension.

Urinary effects

The control period before left atrial distension was compared with the control period after left atrial distension for the variables of urine flow, urine osmolality and free water clearance. There were no significant differences between the two control periods for these variables and thus the values in the two control periods were combined to obtain a single mean control value for comparison with the experimental values. Urine flow increased from a mean control value of 0·85$\pm$0·10 ml./min to a mean experimental value of 1·31$\pm$0·20 ml./min. Urine osmolality decreased from a mean control value of 516$\pm$50·9 m-osmole/kg to a mean experimental value of 379$\pm$45·7 m-osmole/kg. Free water clearance increased from a mean control value of $-0{\cdot}37\pm0{\cdot}11$ ml./min to a mean experimental value of 0·07$\pm$0·07 ml./min. The average time course of these changes in urinary composition which occurred during the 90 min distension of a balloon in the left atrium is shown in Fig. 1. The values in this figure indicate that a positive free water clearance was achieved at a time when the urine was hypertonic. This apparent contradiction is due to the averaging of the results of eighteen tests; in any single experiment free water clearance was positive only when urine was hypotonic.

The average changes in urinary composition which occurred during the first 40 min of atrial distension were qualitatively similar to those reported previously for 30 min tests of atrial distension (Arndt *et al.*, 1963; Ledsome and Mason, 1972). During the first 40 min of atrial distension there was an increase in urine flow, a decrease in urine osmolality, an increase in free water clearance and an increase in osmolal clearance with small increases in sodium and potassium excretion. These changes in the measured renal variables were transient.

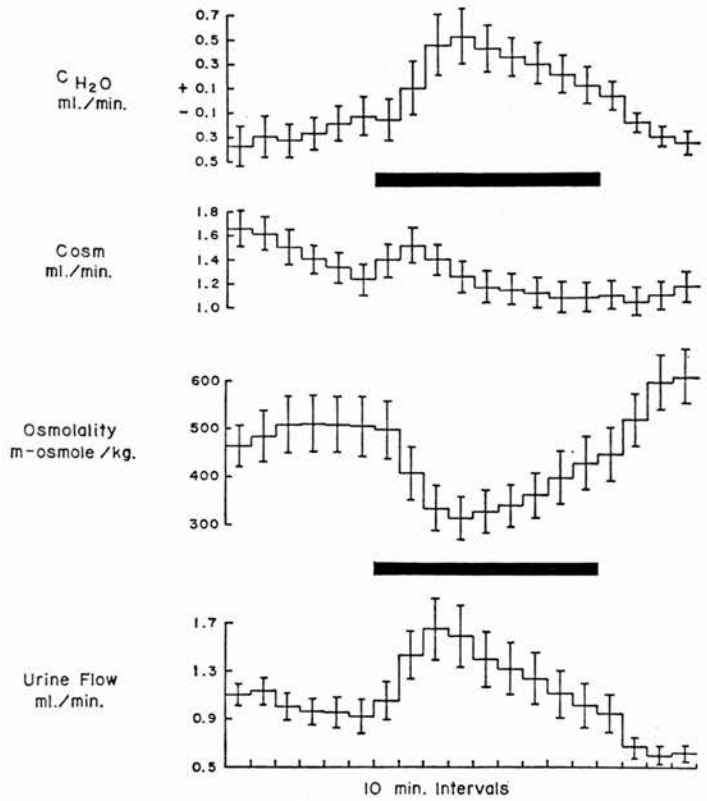

FIG. 1. Effects of 90 min distension of the left atrium on urinary excretion. Each horizontal line in a 10 min interval represents the average value from eighteen tests in thirteen dogs. From above downwards: free water clearance (ml/min), osmolal clearance (ml./min), urine osmolality (m-osmole/kg), urine flow (ml./min). Period of left atrial distension is indicated by the solid bar. S.E. of the mean is indicated by vertical lines.

After starting left atrial distension urine flow usually began to increase during the first 10 min period and reached a maximum rate during the third 10 min period (range, second-seventh 10 min period). The increase in urine flow was always accompanied by a dilution of the urine with a consequent increase in free water clearance. Urine osmolality usually began to decrease during the second 10 min period after left atrial distension and usually reached a minimum during the fourth 10 min period (range, third-seventh 10 min period). Free water clearance usually began to increase during the second 10 min period after left atrial distension and usually reached a maximum rate during the fourth 10 min period (range, second-seventh 10 min period). After the peak change was reached urine flow, urine osmolality and free water clearance began to return to control levels.

Comparison between the final 30 min period of left atrial distension and the mean control values using the Student's *t*-test for paired comparison indicated that the return to control levels was not completed during the period of left atrial distension. The mean value for urine flow during the final 30 min of

distension, 1·12±0·20 ml./min was significantly greater ($P < 0·05$) than the mean control value of 0·85 ml./min. The final mean value for urine osmolality of 395±50·9 m-osmole/kg was significantly less ($P < 0·0025$) than the mean control value of 516 m-osmole/kg. The final mean value for free water clearance of 0·02±0·15 ml./min was also significantly greater ($P < 0·005$) than the mean control value of −0·37 ml./min. Thus there was still a highly significant response to left atrial distension present between 60–90 min after the start of the distension.

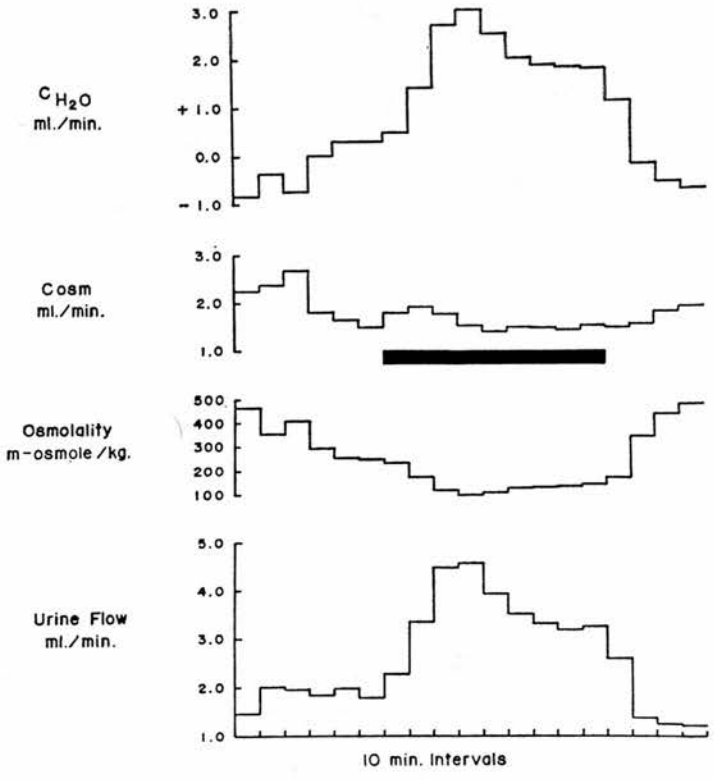

FIG. 2. Effects of a 90 min distension of the left atrium on urinary excretion in one dog. Conventions as in Fig. 1.

An increase in osmolal clearance occurred during eleven of the eighteen tests of left atrial distension. The time course of the increase in osmolal clearance was not similar to the time course of the increase in urine flow (Fig. 1). The increase in osmolal clearance always began in the first 10 min period and usually reached a maximum rate during the second 10 min period (range, first-third 10 min period). Osmolal clearance always returned to control levels by the fourth 10 min period. This time course of the changes in osmolal clearance occurred even during experiments in which large and prolonged increases in urine flow occurred in response to left atrial distension (Fig. 2). In Fig. 2, after starting left atrial distension osmolal clearance increased during the second

10 min period. During the fourth 10 min period osmolal clearance returned to control values, while in the same time interval urine flow, urine osmolality and free water clearance were reaching their maximum change from control levels. Because the increase in osmolal clearance was confined to the first 30 min following the start of left atrial distension, the control and experimental averages for the variables of solute excretion were averaged over the 30 min preceding atrial distension and the first 30 min following the start of left atrial distension respectively. In eleven tests osmolal clearance increased ($P < 0{\cdot}01$) from a control value of $1{\cdot}33 \pm 0{\cdot}16$ ml./min to an experimental value of $1{\cdot}55 \pm 0{\cdot}18$ ml./min, sodium excretion increased ($P < 0{\cdot}1$) from a control value of $62 \pm 13{\cdot}8$ μ-mole/min to an experimental value of $70 \pm 14{\cdot}4$ μ-mole/min and potassium excretion increased ($P < 0{\cdot}01$) from a control value of $58 \pm 8{\cdot}5$ μ-mole/min to an experimental value of $67 \pm 7{\cdot}6$ μ-mole/min. Thus the increase in osmolal clearance constituted an average increase in excretion of 65 μ-osmole/min and of this increase 16 μ-osmole/min (25%) were Na plus anion, 18 μ-osmole/min (28%) were K plus anion, and the remaining 31 μ-osmole/min (47%) were unmeasured solute, e.g. urea. These percentage values were similar to the percentage composition of the solute excretion during the control and experimental periods, when Na plus anion and K plus anion each comprised approximately 30% of the solute excretion and unmeasured solute approximately 40% of the solute excretion. Thus the increase in osmolal clearance which occurred in the eleven tests appeared to be the result of a nonspecific increase in total solute excretion.

The differences in the time course of the increase in solute excretion and the increase in free water clearance are emphasized by examination of the average times taken to reach a maximum change. When atrial distension was continued for a period of 90 min there was an increase in free water clearance which reached a peak at about $37 \pm 4{\cdot}4$ min and which was not complete after 90 min. The increase in osmolal clearance occurred more rapidly, reaching a peak at $14 \pm 2{\cdot}4$ min and being complete by 30 min. These times to peak change in free water clearance and osmolal clearance were significantly different from one another ($P < 0{\cdot}005$).

Discussion

The cardiovascular changes induced by left atrial distension were similar to those which have been reported previously [Ledsome *et al.*, 1961; Arndt *et al.*, 1963; Ledsome and Mason, 1972]. The diuretic responses observed during the first 40 min of left atrial distension were also qualitatively similar to those previously reported in other series [Ledsome *et al.*, 1961; Arndt *et al.*, 1963; Ledsome and Mason, 1972].

Previous investigators have demonstrated that the increase in urine flow associated with left atrial distension was transient despite maintained left atrial distension [Henry *et al.*, 1956; Ledsome *et al.*, 1961; Lydtin and Hamilton, 1964; Shu'ayb, Moran and Zimmermann, 1965]. These investigators all showed that the maximum increase in urine flow occurred after 20–50 min of left

atrial distension. However, in all cases insufficient tests were done to allow conclusions on the time course of the accompanying changes in solute excretion or indeed on whether or not the urine flow did decrease completely to control values if distension was maintained. The results of the present investigation confirm that when atrial distension is prolonged (for 90 min) the decreases in urine osmolality and increases in urine flow, free water clearance and osmolal clearance reach a maximum value and then return towards control values. The results also show that an increase in urine flow and free water clearance may be continued throughout the whole period of atrial distension, and return to control values only after release of the distension. Thus it appears that the diuretic response to left atrial distension may be more prolonged than has been previously supposed.

The suggestion that the diuretic response to left atrial distension is due at least in part to a decrease in the rate of release of antidiuretic hormone from the neurohypophysis [Arndt *et al.*, 1963] is supported by the observations of Johnson, Moore and Segar [1969] who demonstrated that left atrial distension is accompanied by a decrease in antidiuretic activity in the circulating blood. However, the results of Shu'ayb *et al.* [1965] had indicated that the diuretic response was transient despite continued reduction of antidiuretic activity in the blood. This observation cast some doubt on the part played by a reduction of antidiuretic activity in the diuretic response. Recent experiments on anaesthetized dogs by Mason and Ledsome [1971] have shown that when the concentration of infused vasopressin is reduced from a high value to a relatively low value there is a dilution of the urine which is at least partially transient. Although the time course of this latter response, reaching a maximum change in 70 min, was somewhat slower than the response to left atrial distension described in the present experiments, the results of Mason and Ledsome [1971] do provide indirect support for the view that a continued reduction in release of antidiuretic hormone from the neurohypophysis could be associated with a diuretic response which was partially transient.

Shu'ayb *et al.* [1965] argued that the increase in solute excretion during the first 30 min of left atrial distension may be an artefact secondary to the dead space of the urine collecting system. This possibility cannot be ruled out; but any such effects were minimized in the present experiments by excluding those experiments in which the rate of urine flow during the experimental period was less than 0·5 ml./min. Also other experiments [Mason and Ledsome, 1971] have shown that when a diuretic response is induced in an anaesthetized dog by decreasing the rate of an infusion of vasopressin, there is an increase in free water clearance, and either a decrease or no change in osmolal clearance. It therefore seems likely that although the increase in osmolal clearance began rapidly and had a short time course, it is not an artefact of washout.

Arndt *et al.* [1963] provide some support for their suggestion that the increase in osmolal clearance could be due to an increase in glomerular filtration rate associated with a decrease in renal vascular resistance during left atrial distension [Arndt *et al.*, 1963]. This suggestion is indirectly supported by the present and past observation [Ledsome and Mason, 1972] that left atrial

distension is accompanied by a nonspecific increase in osmolal clearance at a time when arterial pressure is decreased. It was also shown [Ledsome and Mason, 1972] that although the increase in free water clearance accompanying left atrial distension could be prevented by infusion of large doses of vasopressin, vasopressin infusion had no effect upon the increase in osmolal clearance. No information is available regarding the mechanism of the proposed haemodynamic change, but indirect evidence, recently presented, indicates that stimulation of cardiac vagal afferents may cause decreases in renal vascular resistance rather than in other vascular beds [Oberg and White, 1970]. More direct evidence implicating left atrial receptors has been presented by Karim, Kidd, Malpus and Penna [1971] who have demonstrated decreased impulse activity in efferent renal nerves during distension of the pulmonary vein-left atrial junctions. Lydtin and Hamilton [1964] demonstrated an increase in renal blood flow and a nonspecific increase in solute excretion which was transient over 30 min in an unanaesthetized dog during prolonged left atrial distension, although in their experiment arterial pressure was also increased. Thus it is possible that the increase in solute excretion during atrial distension may be associated with a reflex decrease in renal vascular resistance.

The results described indicate that the diuretic response to left atrial distension in anaesthetized dogs may be more prolonged than previously described. The time course of the change in osmolal clearance was significantly different from the time course of the increase in free water clearance, and was also unrelated to any differences in the magnitude or time course of the changes in free water clearance in individual experiments (e.g. Fig. 2). The results provide indirect support for the hypothesis that the response depends upon two mechanisms: a decrease in the rate of release of antidiuretic hormone from the neurohypophysis, which may be prolonged; and a haemodynamic mechanism, which under the conditions of the present experiments appears to be short lived.

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council of Canada.

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J. Physiol. (1974), **237**, *pp.* 685–700
With 5 text-figures
Printed in Great Britain

THE RESPONSE TO DISTENSION OF THE PULMONARY VEIN–LEFT ATRIAL JUNCTIONS IN DOGS WITH SPINAL SECTION

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(*Received 4 October 1973*)

SUMMARY

1. A reflex increase in heart rate in response to pulmonary vein distension was observed in decerebrate dogs. This increase could not be totally abolished by treatment with both propranolol and bretylium tosylate. Only bilateral cervical vagotomy abolished the reflex increase in heart rate.

2. A significant increase in heart rate occurred in a total of seventeen spinal dogs during pulmonary vein distension.

3. In seven spinal animals in which blood pressure was maintained by the continuous infusion of noradrenaline, the increase in heart rate could be totally prevented by cervical vagotomy.

4. The time course of the increase in heart rate observed in the spinal animals was rapid, reaching maximum expression within 10 sec of pulmonary vein distension. Such a time course is dissimilar from that associated with pulmonary vein distension in intact or decerebrate dogs in which maximum increases in heart rate take 1–3 min to develop.

5. It is concluded that the reflex tachycardia resulting from pulmonary vein distension may be mediated by both an efferent sympathetic and an efferent vagal pathway, the relative significance of each component being dependent upon the prevailing autonomic drive existent in the animal at any specific time.

INTRODUCTION

Distension of the pulmonary vein-left atrial junctions by means of small balloons has been shown to cause a reflex increase in heart rate (Ledsome & Linden, 1964*b*; Albrook, Bennion & Ledsome, 1972). The nervous pathway for this reflex response has been described as having its afferent limb in the vagus nerves and its efferent limb in the cardiac sympathetic nerves. Justification for the claim that there is no vagal efferent component to this reflex (Ledsome & Linden, 1964*b*; Furnival, Linden & Snow, 1971)

is based on the fact that the increase in heart rate in response to pulmonary vein distension could be abolished or very significantly reduced by cutting both ansae subclavae, or by the administration of propranolol or bretylium tosylate. However, Albrook *et al.* (1972) have shown that in a series of intact and decerebrate dogs a variable but significant component of the reflex increase in heart rate to pulmonary vein distension remained after adequate blockade of β-adrenergic receptors with propranolol.

The present investigation was designed to examine in more detail the possibility that there may be an efferent vagal component to the reflex increase in heart rate induced by pulmonary vein distension. Use of two adrenergic blocking agents and a study of the effects of spinal section on the reflex response allowed a positive conclusion to be reached regarding the existence of an efferent vagal component of the reflex.

METHODS

Mongrel dogs of either sex were given a s.c. injection of morphine sulphate (0·5 mg/kg). One half-hour later, under local anaesthesia (carbocaine 2 %, Winthrop Laboratories), a cannula was inserted into the saphenous vein and general anaesthesia was induced by an intravenous infusion of α-chloralose 0·1 g/kg body wt. (British Drug Houses; 1 g dissolved in 100 ml. of a solution of sodium chloride (0·9 g/100 ml.)). Subsequently a steady state of light anaesthesia was maintained throughout all surgical procedures by the infusion of 10 % of the original dose of chloralose every half-hour.

The dogs were placed on a heated table where oesophageal temperature was kept constant at 37° C ($\pm$ 1° C). A tracheostomy was performed, and the animal artificially ventilated by a respiratory pump (Harvard Inst. Co.; Harvard, Mass.) at a rate of 18 breaths/min with a stroke volume of approximately 50 ml./3 kg body wt. Blood gases were measured from samples of arterial blood. High levels of P_{a,O_2} (100 mmHg) were maintained by the addition of 1 l./min of O_2 to the inspired air. P_{a,CO_2} and pH were measured using standard electrodes (Instrumentation Laboratories) and kept within the normal ranges of 35–40 mmHg and 7·3–7·4 pH units by adjustments of the stroke volume of the respiratory pump or the addition of sodium bicarbonate (1 M) as required. All such corrections were made before the experimental period.

Thoracotomy was performed through the left 5th intercostal space. Once the chest was opened a resistance to expiration of 3 cm H_2O was provided by means of an exhalation valve (Ohio Chemical). Blood volume was expanded at this time by the infusion of a volume of dextran (Travenol, Baxter Laboratories) approximately equal to 10 % of the dog's estimated blood volume (8 % of body weight). The left lung was retracted and small balloons placed in each of three pulmonary veins after the manner of Ledsome & Linden (1964*b*). The left lung root was then tied off completely with stout cord.

The femoral arterial pressure was recorded through a 15 cm length of Teflon tubing (1 mm bore) connected to a strain gauge manometer (Statham Inst. Co., Inc., Puerto Rico, Model P23Gb). The frequency response of such a system as tested by the method of Hansen (1949) was flat to better than 35 Hz ($\pm$ 5 %). After amplification (Honeywell, Accudata 113), pressure was recorded on a direct writing ultraviolet light recorder (Honeywell, Visicorder 1508) and could be depicted as pulsatile

pressure or as mean pressure, obtained by electrical integration. The electrocardiogram was recorded from bipolar chest leads and after preamplification (Grass Instrument Co., P15) was displayed on the ultra-violet light recorder. Heart rate was recorded from a cardiotachometer (Honeywell, Accudata 103) triggered by the R wave of the electrocardiogram. The response time of the cardiotachometer system was 2 sec. All heart rates used in the results were counted from the electrocardiogram over periods of 1 min except when transient changes were reported.

Two groups of dogs were used in these experiments, those made decerebrate, and those made spinal. In the decerebrate group, after placement of the pulmonary vein balloons, five dogs of 8–12 kg were turned into the prone position and their heads placed in a stereotaxic frame (Precision Cinematographique, Paris). Although the shapes of the heads varied, the position of the head was standardized by placement of the ear bars (the 0 reference) and by tilting the head downward so that the eyes were in the same plane as the ear bars. This position was secured by mandibular clamps and a face plate. The dogs were made decerebrate at the midcollicular plane by a high frequency coagulation system previously described (Albrook *et al.* 1972). In the other group, seventeen dogs of wider weight range (8–18 kg) were made spinal by a high cervical transection of the spinal cord. The animals were positioned in the stereotaxic frame as above for easy access to the dorsal neck region. After dissection and retraction of the muscles overlying the foramen magnum and the first two cervical vertebrae, the dura over the cord was opened through the foramen and the cerebrospinal fluid allowed to escape. The dura was then removed. When spinal section was required during the course of the experiment, the cord was carefully severed at the level of the first cervical vertebra by a blunt spatula, taking care to avoid the basilar artery ventrally. To prevent undue movement during this procedure, short-term paralysis was induced by the use of succinyl choline 0·5 mg/kg (E. S. Squibb & Sons; Sucostrin chloride) just prior to sectioning the cord. Bleeding was controlled by packing the wound lightly with Gelfoam (Absorbable Gelatin Sponge, Upjohn, Don Mills). In both groups of dogs (decerebrate and spinal), care was taken to ensure a continued steady level of anaesthesia (by the addition of 10 % of the original dose of chloralose every half hour) before, during and after section of the brain or cord.

In the spinal animals it was necessary to preserve adequate blood pressure by the continuous infusion of noradrenaline (Levophed bitartrate injection, U.S.P., Winthrop). This was carried out in seven dogs through a cannula inserted into the right external jugular vein attached to a variable speed constant infusion pump (Harvard Instruments). The dose required to maintain mean femoral blood pressure at about its value before transection of the cord (120 mmHg) varied with the individual animal, but was within the range of 1–2 μg/kg.min delivered in a volume of from 0·5 to 1 ml./min.

In all other cases where drugs were used, viz. isoprenaline (K & K Laboratories Inc.; Isoprenaline salt sulphate), propranolol (Ayerst Lab; Ay-64043), and bretylium tosylate (Burroughs Wellcome & Co.; Darenthin) they were administered by rapid injection via the saphenous vein cannula and followed by a 5 ml. wash of saline.

Experimental protocol. After completion of all surgical procedures, when a normal acid-base balance and a cardiovascular steady state had been achieved, the experimental period began. Testing for the appearance of a reflex response to pulmonary vein distension was always done in the same manner. Following a control period, the pulmonary balloons were inflated with 0·5–1 ml. saline and distension maintained until a steady state had been reached. The balloons were then deflated and a second control recorded when a further steady state had been reached. Changes in the heart rate and blood pressure were always calculated from the difference between

steady-state inflation values and the mean of the control periods before and after inflation. Time to reach the steady-state values during the after balloon inflation varied considerably between the decerebrate and spinal animals. Thus intact and decerebrate trials were calculated from steady-state changes 2–3 min after balloon inflation, and 2–3 min after balloon deflation. Changes in spinal animals, which displayed a more rapid response time and were in addition less stable, were calculated from steady-state values within 2 min of balloon inflation or deflation. Recording periods were always at least 1 min in length. When transient changes were examined heart rates were counted over 10 sec periods.

During the course of the experiment, testing for the reflex response was done in intact, decerebrate and spinal animals and after various drug regimens. The intact series comprised animals prepared for section of brainstem or cord with the dura open over brain or cord. Decerebrate tests included any tests performed in decerebrate animals in which no other physiological or pharmacological interventions had taken place. Spinal tests were started only after the initial profound trauma of spinal section had subsided and the noradrenaline infusion had stabilized mean arterial pressure at a level similar to that present before spinal section (usually 20 min–1 hr post section).

The degree of blockade of cardiac β-adrenergic receptors induced by propranolol was assessed by observing the maximum change in heart rate induced by rapid intravenous injection of isoprenaline (0·5 mg/kg). Blockade was considered adequate if the increase in heart rate was reduced by at least 95 % over the increase in heart rate observed before administration of propranolol. Testing of blockade in this manner was carried out before and after each test of pulmonary vein distension.

The significance of the changes in heart rate and arterial pressure was tested using a t test for paired data with a Student's range. Because it was not known whether increases or decreases in these variables would occur this represents a two-tailed test and values for probability are quoted as $2P$. For purposes of comparison of the changes in heart rate and arterial pressure before and after an intervention such as spinal section or drug administration, average changes for each dog in each state were used for paired comparisons. The averages for each dog were calculated from at least three tests of pulmonary vein distension in each state.

RESULTS

Effects of pulmonary vein distension in the decerebrate dog. As previously described, the dog made decerebrate by the high frequency coagulation system displays a remarkably stable cardiovascular state. Following an initial decrease in heart rate and blood pressure upon decerebration, there is little change except during experimental intervention. The decrease in heart rate appears to be of vagal origin, and a high vagal tone prevails with brisk cardiovascular reflexes. Inflation of the small balloons in the pulmonary veins under these conditions causes a reproducible increase in heart rate without significant change in arterial blood pressure (Albrook *et al.* 1972).

The results to be presented here concern a series of tests carried out in five decerebrate dogs treated with two sympathetic blocking agents in an effort to ensure complete sympathetic blockade of the efferent pathway of the reflex response to pulmonary vein distension. The mean values for

heart rate and arterial pressure in these animals when decerebrate are given in Table 1. In fifteen trials of pulmonary vein distension in the decerebrate state there was always an increase in heart rate. Propranolol (0·5 mg/kg I.V.) given in a dose sufficient to block > 95% of the tachycardia resulting from β-receptor stimulation by isoprenaline (0·5 μg/kg) decreased heart rate, but did not affect the mean arterial pressure. After

TABLE 1. The effects of decerebration, sympathetic blockade and cervical vagotomy on the reflex response to distension of the pulmonary vein–left atrial junctions. Each set of figures is for fifteen tests in five dogs and shows mean, S.E. of mean and range

	Heart rate beats/min		Arterial pressure mmHg	
	Control	Change on distension	Control	Change on distension
Intact	141 ± 5·9 103 to 168	+26 ± 6·2 5 to 76	117 ± 2·2 100 to 133	−5 ± 0·8 −10 to 2
Decerebrate	107 ± 10 58 to 196	+15·7 ± 2·2 2 to 27	105 ± 1·3 94 to 112	+2·9 ± 2·2 −8 to 21
Decerebrate + propranolol	85 ± 9 42 to 158	+5·9 ± 1·6 −1 to 16	106 ± 2·7 87 to 118	+3·9 ± 1·9 −5 to 20
Decerebrate + propranolol + bretylium	68 ± 7 32 to 130	+6·3 ± 0·7 2 to 13	123 ± 1·9 104 to 136	+3·8 ± 1·2 −2 to 14
Above plus vagotomy	107 ± 7·9 96 to 144	0 ± 0·2 −2 to 1·5	127 ± 3·2 111 to 145	−9·1 ± 2·1 −24 to 1

administration of propranolol the reflex increase in heart rate caused by pulmonary vein distension was significantly reduced ($2P < 0·025$) but not abolished.

When bretylium tosylate (10 mg/kg) was administered to these animals to block post-ganglionic sympathetic nerves, mean heart rate decreased and blood pressure increased by about 20 mmHg (Table 1). The response to pulmonary vein distension was, however, not significantly altered. Arterial pressure changes during distension of the pulmonary vein-atrial junctions in the decerebrate, β-blocked, and bretylium treated situations were small and not statistically significant (Table 1).

Bilateral cervical vagotomy totally abolished any increase in heart rate upon pulmonary vein distension in these animals. Vagotomy brought about an increase in heart rate to an average value of 107 beats/min, but

did not alter arterial pressure. The heart rate after treatment with propranolol and bretylium and after vagal section was slower than expected. Inflation of the pulmonary balloons in these vagotomized dogs caused no change in heart rate, but decreased blood pressure by an average of 9 mmHg ($\pm 2{\cdot}0$) in fifteen trials. This fall in pressure was significantly different from the pressure changes in the decerebrate, β-blocked and bretylium treated state ($2P < 0{\cdot}10$). Changes in heart rate and mean arterial pressure in the individual tests are shown in Fig. 1.

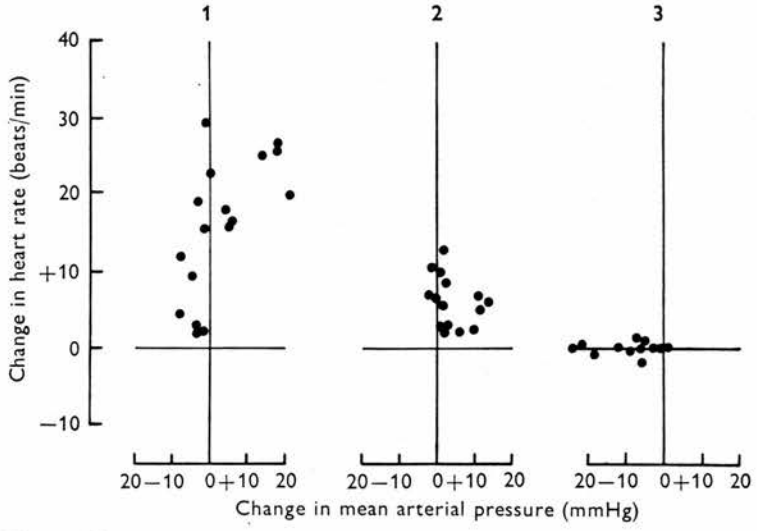

Fig. 1. Changes in heart rate and mean arterial pressure during distension of the pulmonary vein–left atrial junctions in individual tests in five dogs. 1, decerebrate animals. 2, decerebrate plus propranolol (0·5 mg/kg) and bretylium tosylate (10 mg/kg). 3, as in 2, after cervical vagotomy.

Effects of pulmonary vein distension in the spinal animal. To determine whether a reflex increase in heart rate remained after complete surgical elimination of efferent sympathetic pathways, a second series of experiments was conducted in which spinal transections at the level of the first cervical vertebra were performed. In ten spinal dogs there was an increase in heart rate (mean value of 6 beats/min; range 0–27) during pulmonary vein distension, but the progressive fall in arterial pressure and increase in heart rate following spinal section prevented any careful analysis of its characteristics. These results are therefore not reported in detail here. A group of seven dogs in which the spinal cord was transected were treated with a continuous infusion of noradrenaline (1–2 μg/kg.min) to maintain systemic blood pressure close to the pressure prior to section. Mean values

of heart rate and arterial pressure in these animals before and following section of the cord and the infusion of noradrenaline are shown in Table 2. Inflation of the pulmonary balloons in the intact state, before spinal section, caused a mean increase in heart rate of 19 beats/min and a small but not significant decrease in arterial pressure. In the same animals after spinal section pulmonary vein distension caused an increase of 14 beats/min which was not significantly different from the control value. However, two characteristics of the response in the spinal animals were significantly different from the response in the intact animals, the time course of the reflex response, and the appearance of a small increase in arterial pressure with the increase in heart rate which was significantly different ($2P < 0{\cdot}10$) from the changes in the control state (Table 2).

TABLE 2. Changes in heart rate and mean arterial pressure induced by distension of the pulmonary vein–left atrial junctions. Each set of figures is for twenty-one tests in seven dogs and shows mean, S.E. of mean and range. Results for intact animals, after spinal section and infusion of noradrenaline and after cervical vagotomy (noradrenaline infusion continued)

	Heart rate (beats/min)		Arterial pressure (mmHg)	
	Control	Change on distension	Control	Change on distension
Intact	141 ±8 49 to 242	19 ±5 4 to 37	126 ±3·4 102 to 160	−2 ±0·8 −8 to 4·7
Spinal + noradrenaline	83 ±4 60 to 156	14 ±2 2 to 48	129 ±2·9 98 to 158	2·6 ±0·8 −1·2 to 8
Spinal + noradrenaline + vagotomy	184 ±10 108 to 264	−1·5 ±0·7 −8 to 0·7	130 ±10·4 66 to 216	−13 ±3 −40 to 0

In an intact or decerebrate dog, pulmonary vein distension typically causes an immediate increase in heart rate, followed by a slow continuous rise until a steady state is reached 1·5–3 min later. Deflation of the pulmonary balloons results in a similarly slow decline towards initial control values. The reflex response to pulmonary vein distension in the spinal dog was different. Inflation of the balloons caused an immediate increase in heart rate which did not increase further despite continued inflation. Deflation was followed by a rapid return to control levels of heart rate. A study of the time course of the appearance of the increase in heart rate at pulmonary distension in twenty-four trials in these seven dogs, while intact, revealed that of the maximum response achieved after 2 min 49 %

appeared within 10 sec of balloon inflation. The rate of disappearance of the increase in heart rate at balloon deflation was similar, a 37 % reduction occurring within 10 sec. In these same seven animals after spinal section, in twenty-one trials of balloon inflation 100 % of the reflex increase in heart rate appeared within 10 sec, while 80 % of it disappeared 10 sec after deflation. A typical response in a spinal animal is shown in Fig. 2, and the average changes in the twenty-one tests are plotted in Fig. 3. Differences in the changes in arterial pressure on inflation in the intact and spinal animals are also apparent in Fig. 3. It will be noted that in the intact animals there was a transient fall in arterial pressure at 10–20 sec after balloon inflation. This occurred in three of the seven animals and has been

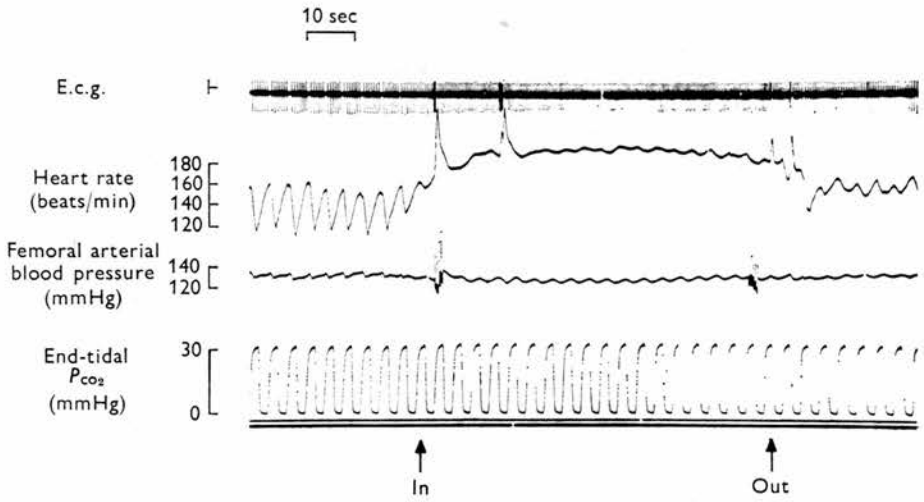

Fig. 2. An example of the effects of distension of the pulmonary vein–left atrial junctions in a spinal dog. Distension started at arrow 'in', stopped at arrow 'out'. From above downwards, electrocardiogram, heart rate, femoral arterial pressure in which two short periods of pulsatile pressure are recorded and end-tidal P_{CO_2}.

described previously (Carswell, Hainsworth & Ledsome, 1970; Ledsome & Hainsworth, 1970). There was no such transient fall in arterial pressure in the animals after spinal section in which the arterial pressure increased at the same time as the increase in heart rate. It was also observed that in these spinal animals bilateral carotid occlusion caused an increase in heart rate and in mean arterial pressure. In ten tests in five spinal animals carotid occlusion maintained for 20 sec caused an increase in heart rate of 14·8 (S.E. of mean ± 3·8) beats/min and an increase in arterial pressure of 9·9 (S.E. of mean ± 2·5) mmHg. A record of one such test is shown in

Fig. 4. In these tests the changes in arterial pressure were directly dependent upon the increase in heart rate.

Cutting the vagus nerves in the spinal animals caused a large increase in heart rate but no change in mean arterial pressure although the range

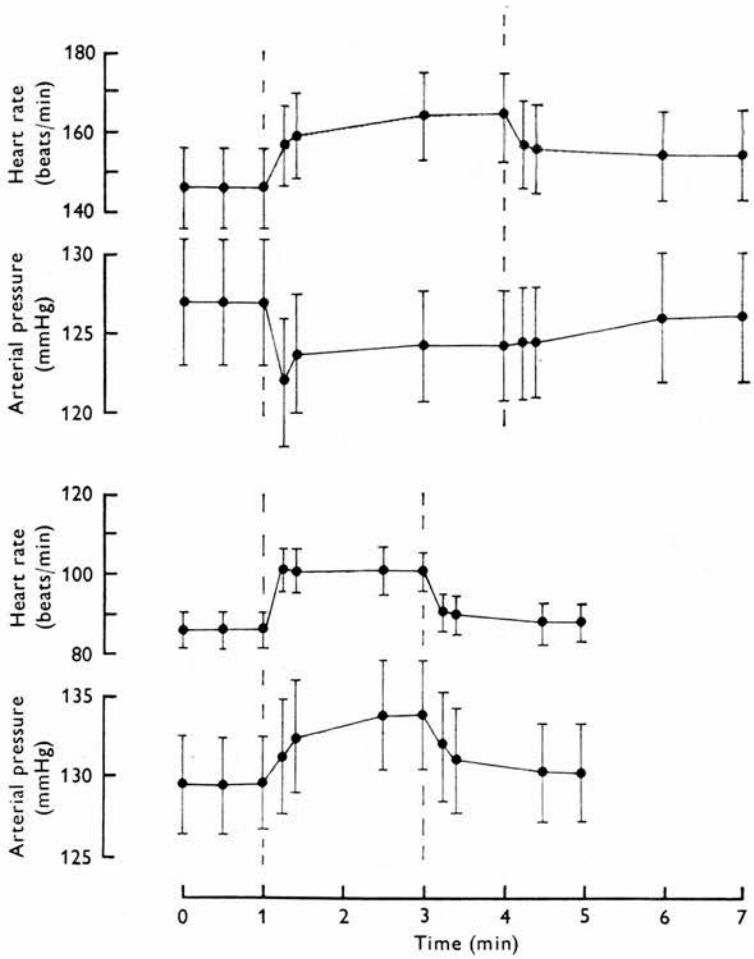

Fig. 3. Heart rate and arterial pressure before, during and after distension of the pulmonary vein–left atrial junctions. Each point represents the average (± S.E. of mean) of twenty-one tests in seven dogs. Upper two traces are tests in intact anaesthetized animals, lower, two traces in the same animals after high spinal section and during infusion of noradrenaline.

of pressures was increased. There was no significant change in heart rate in response to pulmonary vein distension after vagotomy. However, there was a fall in arterial pressure during pulmonary vein distension (Table 2).

This change was significantly different from the arterial pressure changes observed in the spinal dogs ($2P < 0{\cdot}10$). The changes in individual tests are plotted in Fig. 5. It may be noted that much of the apparently large average fall in arterial pressure was due to the fact that in each of three tests in one dog there was a fall in arterial pressure of 45–50 mmHg on

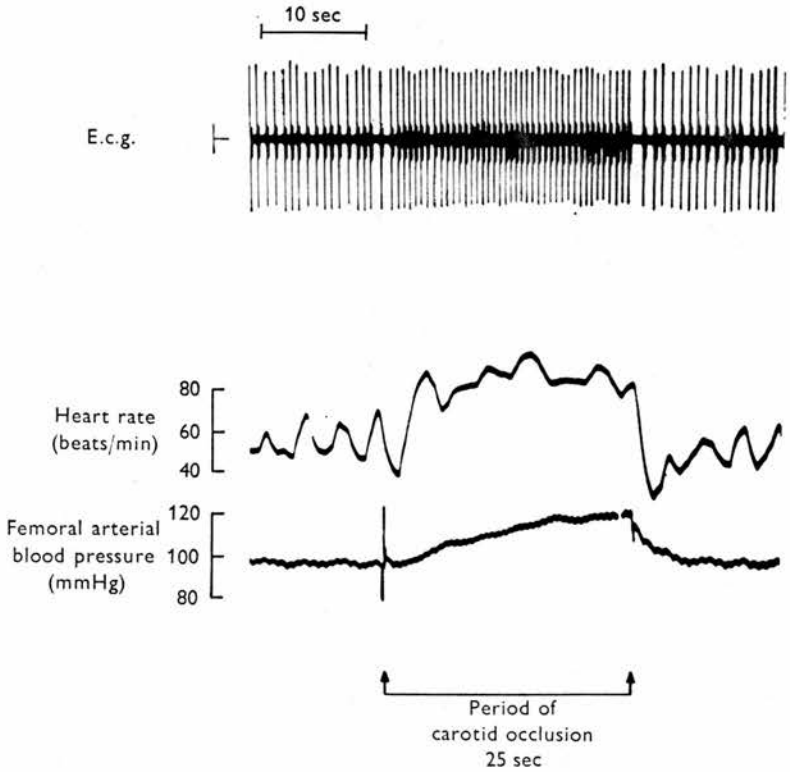

Fig. 4. A record of the effect of carotid occlusion in a dog after high spinal section and during infusion of noradrenaline. From above downwards: electrocardiogram, heart rate and mean arterial pressure. Bilateral carotid occlusion during the period shown.

pulmonary vein distension. The heart rate in this animal was 264 beats/min after vagotomy. Before vagotomy this animal had a heart rate of 90 beats/min and had consistently shown an increase in heart rate of 24 beats/min and an increase in mean arterial pressure of 6 mmHg on pulmonary vein distension.

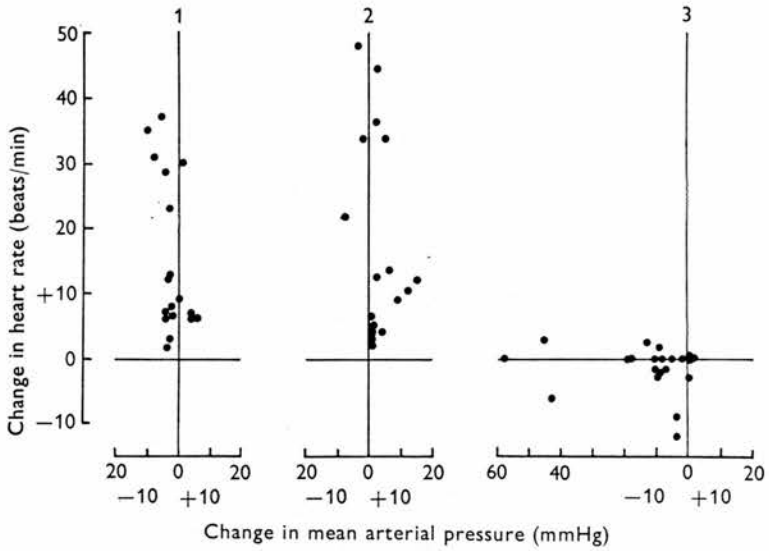

Fig. 5. Changes in heart rate and mean arterial pressure during distension of the pulmonary vein–left atrial junctions in individual tests in seven dogs. 1, intact animal. 2, after high spinal section during infusion of noradrenaline. 3, as in 2 after cervical vagotomy.

DISCUSSION

Complex unencapsulated nerve endings (atrial receptors) have been demonstrated in the atrial subendocardium and are concentrated at the entrances of the veins into the atria (Nonidez, 1937; Coleridge, Hemingway, Holmes & Linden, 1957). Distension of the region of the junctions of the pulmonary veins with the left atrium, by means of small balloons, has been shown to cause intense stimulation of left atrial receptors (Kidd, Ledsome & Linden, 1966) and to be associated with a reflex increase in heart rate (Ledsome & Linden, 1964*b*). Distension of an isolated pouch of the left atrium also causes stimulation of atrial receptors and a reflex increase in heart rate (Ledsome & Linden, 1967). The afferent path of the reflex is in the vagus nerves and the efferent path has been thought to be solely in the cardiac sympathetic nerves (Ledsome & Linden, 1964*b*; Furnival *et al.* 1971). Because earlier findings (Albrook *et al.* 1972) had indicated the possibility of unexpected variation in the efferent pathway of the reflex response to pulmonary vein distension, the experiments reported here were carried out to clarify this point. The fact that propranolol (0·5 mg/kg) did not completely abolish any increase in heart rate on pulmonary vein distension in either the intact or decerebrate dog had previously been attributed to two possibilities. First as a competitive antagonist, 100 % antagonism might not be expected, and secondly the

use of isoprenaline as an index of the degree of β-antagonism might be unreliable. Despite the fact that the dose of propranolol used was able to block more than 95 % of the heart rate response to isoprenaline stimulation of β-receptors an extremely wide range of percentages (0–100 %) of the control response to pulmonary vein distension remained after propranolol. Although in absolute terms the average increase in heart rate on pulmonary vein distension was small after administration of propanolol it nevertheless represented a significant change. It is unlikely that the increases remaining are simply the result of incomplete β-antagonism, unless the ability of propranolol to block isoprenaline induced β-stimulation is considerably better than its ability to block normal noradrenaline mediated nerve traffic. It has been clearly demonstrated that this is not the case. Indeed propranolol in all doses tested is at least as effective an antagonist of sympathetic excitation of the heart by either noradrenaline (0·0005–50 μg/kg) or direct nerve stimulation (0·25–15 Hz; 15 V; 2 msec) as it is of isoprenaline (0·005–5 μg/kg) induced β-excitation (Ledsome, Kellett & Burkhart, 1974). Thus the suppositions used to explain the inadequacy of propranolol as a β-antagonist, useful for distinguishing sympathetically mediated responses from parasympathetically mediated responses, appear to be invalid.

The present results in decerebrate dogs treated with both propranolol and bretylium tosylate can only support the contention that the increase in heart rate in response to pulmonary vein distension which remains after β-blockade is not of sympathetic origin. Bretylium tosylate has been shown to produce effective blockade of cardiac sympathetic nerves without interfering with cardiovascular reflexes mediated through the vagus nerves (Ledsome & Linden, 1964*a*). If the reflex increase in heart rate had been simply a result of random impulses defying the β-blockade by successful competition with propranolol, one would expect the post-ganglionic interference of bretylium to prevent any increase. However, the amount of the reflex remaining after propranolol in these dogs, though small, was not altered by the addition of bretylium tosylate to the preparation.

Some comment is required on the use of decerebrate animals and on the condition of the animals when both propranolol and bretylium tosylate were present. The abnormally low heart rate following vagotomy and the administration of propranolol and bretylium tosylate (mean 107 beats/min) indicates that in combination these two drugs are exerting a curious effect on the heart. In the anaesthetized dog section of the cardiac sympathetic nerves or the administration of propranolol together with cervical vagotomy or atropinization usually leads to a heart rate of about 140 beats/min (e.g. Edis, Donald & Shepherd, 1970). Bretylium in varying concentrations can act as an anticholinesterase, anticholinergic or antimonoamine oxidase

agent (Ferry & Morris, 1971; Furchgott, Sanchez-Garcia, Wakade & Cervoni, 1971). The predominant effect depends on the concentration at the cell. Because it is not known what concentrations are reached in cardiac muscle (Boura & Green, 1965) it is not possible to predict which if any of these mechanisms might be present. However, there is no doubt that after administration of bretylium there is a high vagal tone, possibly originating partially reflexly as a consequence of the raised arterial pressure which is usually present for 1–2 hr following administration of bretylium (Ledsome & Linden, 1964*a*). Nevertheless, high vagal tone cannot be used as an explanation of the slow heart rate after administration of bretylium and cervical vagotomy and this bradycardia remains unaccounted for.

The decrease in mean arterial pressure which occurred on distension of the pulmonary vein–left atrial junctions following vagotomy is of more immediate concern. Because both vagal and sympathetic efferent systems had been eliminated it could be due only to either an obstruction to ventricular filling or to some other local effect leading to a decrease in cardiac output. It is true that the preparation at this stage has a somewhat unstable cardiovascular response to any imposed change. Previous measurements of left atrial pressure, pulmonary arterial pressure and right atrial pressure during distension of the pulmonary vein–left atrial junctions (Ledsome & Linden, 1964*b*) have indicated that there was no obstruction to blood flow through the left atrium. It is possible that the prone position of these animals in contrast to the supine position used in previous experiments predisposed to a minor degree of obstruction to blood flow through the left atrium. However, it is unlikely that the reflex increase in heart rate which occurred in response to pulmonary vein distension before vagotomy was secondary to a decrease in cardiac output and consequent decrease in arterial baroreceptor stimulation, as the increase in heart rate was accompanied by a small increase in mean arterial pressure (Table 1).

The elimination of the descending efferent sympathetic system by high transection of the spinal cord forces us to assign any cardiovascular reflex observed to a vagal, local or spinal mechanism. Reflex increases in heart rate have been demonstrated in spinal cats during stimulation of afferent cardiac sympathetic nerve fibres (Malliani, Parks, Tuckett & Brown, 1973). However, the appearance in the spinal dogs of a response to pulmonary vein distension which was totally abolished by cervical vagotomy is strong evidence in favour of a vago-vagal component. The alteration in the time course of the response indicates the removal by spinal section of a slow component and provides additional evidence that the remaining component is mediated through efferent vagal fibres. The small changes

in mean arterial pressure which accompanied the increases in heart rate were altered by spinal section (Fig. 3). The occurrence of a transient decrease in arterial pressure has been described previously (Ledsome & Hainsworth, 1970; Carswell *et al.* 1970) and these changes are dependent upon a decrease in sympathetic vasoconstrictor tone. Thus it is not surprising that this effect was eliminated by spinal section. The small increase in mean arterial pressure which occurred in most tests after spinal section was probably secondary to an increase in cardiac output brought about by the increase in heart rate from a relatively slow initial rate. It may be noted that in this preparation carotid occlusion also provided an increase in arterial pressure which was directly proportional to the increase in heart rate. After vagotomy in this group, as in the previous group of experiments, distension of the pulmonary vein-left atrial junctions caused a decrease in mean arterial pressure. It has been noted that in the animal in which the largest changes occurred the heart rate was extremely rapid, presumably secondary to the noradrenaline infusion. It may be that with the small heart which would be expected in this state, distension of the pulmonary vein balloons may have occupied a significant volume of the left atrium and interfered with left ventricular filling.

It should be emphasized that the small changes in arterial pressure observed in these experiments were in no way comparable in magnitude to those described by Edis *et al.* (1970). They used larger balloons in the pulmonary veins and may have been stimulating receptors other than those stimulated in the present experiments (for discussion of this point see Furnival *et al.* 1971). There was never in the present experiments a reflex slowing of the heart (Figs. 3 and 5). Edis *et al.* (1970) claimed that pulmonary vein distension could be accompanied by a reflex bradycardia mediated through an efferent vagal pathway; the present results are in direct opposition since they demonstrate a reflex increase in heart rate partly mediated through an efferent vagal pathway but mainly through an efferent sympathetic pathway.

In a recent review Paintal (1973) has suggested that differences between the results of Edis *et al.* (1970) and previous experiments of the type reported here (e.g. Ledsome & Linden, 1964*b*) could be accounted for on the basis that Edis *et al.* (1970) recorded maximal changes in heart rate whereas Ledsome & Linden (1964*b*) recorded only steady-state changes. The argument is untenable; the time course of the reflex response to distension of the pulmonary vein–left atrial junction was described in the original report (Ledsome & Linden, 1964*b*) and the initial changes in heart rate and arterial pressure were discussed in detail in two later publications (Ledsome & Hainsworth, 1970; Carswell *et al.* 1970). It is apparent from all of these descriptions as well as the results of the present investigation

(Fig. 3) that not even a transient bradycardia has ever been observed on distension of the pulmonary vein–left atrial junctions using the technique of Ledsome & Linden (1964*b*). There is therefore no basis for the conclusion (Edis *et al.* 1970; Paintal, 1973) that stimulation of atrial receptors may cause either a reflex tachycardia or bradycardia, the response depending upon the initial heart rate.

Two methods have been used in the present experiments to completely eliminate the efferent sympathetic pathway to the heart: total pharmacological blockade and high spinal section. The results make it reasonable to suppose that there may be an efferent vagal component to the reflex increase in heart rate which accompanies distension of the pulmonary vein–left atrial junctions. Former evidence (reviewed by Linden, 1972) certainly points to the existence of an efferent sympathetic pathway to the reflex and the present experiments do not in any way detract from that conclusion. However, previous evidence (Ledsome & Linden, 1964*b*) utilizing sympathetic blocking agents does not completely preclude the possible existence of a vagal component to the efferent pathway. It appears that there may exist two separate efferent pathways for increasing heart rate upon stimulation of left-atrial receptors by pulmonary vein distension. Usually the sympathetic pathway is predominant. However, the relative contributions of either pathway to the total reflex response may depend upon the autonomic drive to the heart at that particular time.

This work was supported by the Medical Research Council of Canada and the British Columbia Heart Foundation.

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Effects of Obstruction of the Mitral Orifice or Distention of the Pulmonary Vein–Atrial Junctions on Renal and Hind-Limb Vascular Resistance in the Dog

By **James M. Mason and John R. Ledsome**

ABSTRACT

This investigation attempted to determine whether stimulation of intrathoracic receptors, including those in the left atrium, is associated with changes in renal vascular resistance. In 19 dogs, the left kidney was perfused at constant pressure and the right hind limb was perfused at constant flow. A partial obstruction of the mitral orifice that increased left atrial pressure by less than 20 cm H_2O caused tachycardia, hypotension, renal vasodilatation (5% increase in flow), and hind-limb vasoconstriction (10% increase in pressure). A partial obstruction of the orifice that raised left atrial pressure more than 20 cm H_2O caused tachycardia, hypotension, and more hind-limb vasoconstriction (20% increase in pressure), but renal vascular resistance did not change. After bilateral vagotomy in either the thorax or the neck, partial obstruction of the mitral orifice caused constriction in both the renal and the hind-limb vascular beds. In 10 dogs, localized distention of three pulmonary vein-left atrial junctions caused an increase in heart rate and a small but significant increase in renal blood flow but had no effect on hind-limb vascular resistance. At least a part of the reflex renal dilatation caused by mitral obstruction probably resulted from stimulation of left atrial receptors.

KEY WORDS atrial receptors vagotomy renal blood flow cardiovascular reflexes heart rate intrathoracic receptors

■ Morphological studies and electrophysiological recordings have established the presence of sensory receptors in the cardiopulmonary region whose afferent fibers travel in the vagus nerves (1). Recent investigations into the function of cardiopulmonary receptors have involved interruption and stimulation of cardiac vagal afferents in anesthetized cats (2) and interruption of cardiac vagal afferents during hemorrhage in anesthetized cats (3) and dogs (4). The results indicate that stimulation of receptors in the cardiopulmonary area whose afferent fibers are in the vagus nerves might have more profound effects on renal vascular resistance than it does on other vascular beds. Observations of the effects of stimulation of left atrial receptors (5) have shown that such stimulation is accompanied by a decrease in renal sympathetic nerve activity with no change in lumbar or splenic nerve activity. However, it has not yet been shown directly that the observed changes in sympathetic nerve activity are indeed transformed into changes in renal vascular resistance.

The present investigation attempted to determine whether stimulation of left atrial receptors is associated with changes in renal vascular resistance. Two techniques were used. The steady-state vascular response of the kidney was compared with that of the hind limb during graded obstruction of the mitral orifice. This technique produces left atrial distention and stimulation of left atrial receptors (6); however, it also distends the whole pulmonary vascular bed and does not localize the stimulus to the left atrial receptors. Therefore, the effect of distention of the pulmonary vein–left atrial junctions (7) on renal and hind-limb vascular resistance was tested. Distention of the pulmonary vein–left atrial junctions provides a powerful stimulus to left atrial receptors (8) but does not interfere with blood flow through the left atrium.

Methods

Dogs (16–24 kg) were injected with morphine sulfate (0.5 mg/kg, sc). One hour later under local anesthesia (mepivacaine hydrochloride 1%) a catheter was inserted through a saphenous vein into the inferior vena cava, and each dog was anesthetized by infusing chloralose (0.1 g/kg, iv) dissolved to make a solution of 1 g chloralose/100 ml sodium chloride solution (0.6 g/100 ml). Subsequently, during the experimental procedures, a steady state of light anesthesia and fluid input was maintained by the constant infusion of a 0.5% chloralose solution (0.5 g chloralose/100 ml 0.9% sodium chloride) into the left external jugular vein; the solution was

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This study was supported by the Medical Research Council of Canada.

Received November 27, 1973. Accepted for publication April 4, 1974.

delivered by a motor-driven syringe pump (Harvard) at a rate of approximately 1.0 ml/min.

As soon as possible after the induction of anesthesia a tracheostomy was performed, and artificial respiration was started with a mixture of 40% oxygen in air supplied by a respiration pump (Harvard model 614); the rate (about 18/min) and stroke (about 16.6 ml/kg body weight) of the pump were adjusted to approximately equal the parameters of the dog's spontaneous respiration. When the chest was opened, a resistance to expiration equivalent to 3 cm H_2O was provided by an exhalation valve. At intervals during the experiment, samples of arterial blood were taken and pH, Pco_2, and Po_2 were measured with electrodes (Instrumentation Laboratories model 113-51). Adjustments were made to the respiratory pump or small infusions (10-20 mEq) of sodium bicarbonate solution (1M) were given to maintain arterial Pco_2 between 35 mm Hg and 40 mm Hg and pH within the range of 7.3 to 7.4; no adjustments were made during the control or experimental periods.

The left side of the chest was opened in the fourth intercostal space. Hemostasis was achieved using electrocautery with careful coagulation of all bleeding points in any area of surgery. A large balloon was placed in the left atrial appendage as described previously (9), and a small balloon was placed in each of three left pulmonary veins (7). The subclavian artery was cleared, and two loose ligatures were placed around it. The femoral artery was exposed in the right leg, and two loose ligatures were placed around it.

With the dog lying on its right side, a left flank incision was made to approach the abdominal aorta. Careful dissection across the retroperitoneal space avoided any tears in the peritoneum. A section of the aorta was cleared approximately 5 cm below the origin of the renal arteries, and a loose ligature was placed around it. A loose ligature was placed between the origin of the right and the left renal arteries. Between these two ligatures there were usually two pairs of lumbar arteries which were individually isolated, ligated proximally and distally, and transected. A brachial artery was cannulated for systemic pressure measurements.

The subclavian artery was then cannulated. The cannula (8 mm, o.d.) consisted of a bifurcated polyvinyl tube; one branch of the tube passed through a roller pump to perfuse the right hind limb and the second branch passed through a modified roller pump to perfuse one kidney. The extracorporeal pump system was primed with 50 ml of 0.9% saline. The right femoral artery was cannulated, and the flow rate of the pump was immediately adjusted to keep the perfusion pressure of the limb approximately equal to arterial blood pressure. The abdominal aorta was cannulated with the end of the cannula pointing rostrally and the modified roller pump was started (Fig. 1). The roller pump that perfused the kidney had been modified to allow perfusion at a constant pressure. The mean renal perfusion pressure signal was compared with a voltage set by a potentiometer, and the difference was amplified and inverted to drive the pump motor. The perfusing pressure could then be set to any predetermined level; usually it was set close to systemic arterial blood pressure.

The loose ligature between the two renal arteries was then tightened; the pump continued to perfuse the aortic pouch and the kidney at approximately arterial blood pressure. In some dogs, the right and left renal arteries were too close together to permit a ligature to be tied between them. In such cases one renal artery was tied off and a ligature was tied around the aorta above both renal arteries.

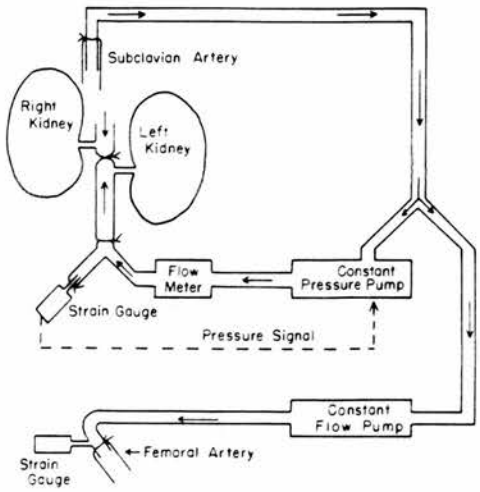

FIGURE 1

Diagram of the major components of the circuit for perfusion of the hind limb at constant flow and the kidney at constant pressure.

The hind limb was perfused at constant flow to negate any effects resulting from hypoxia during periods of reflex vasoconstriction. The kidney was perfused at constant pressure, because preliminary experiments had demonstrated that increasing sympathetic activity to a kidney perfused at constant flow could cause a greatly increased, prolonged change in renal perfusion pressure from which renal vascular resistance failed to return to control values. These increases in perfusion pressure occasionally exceeded 300 mm Hg; they can be accounted for by the shape of the pressure-flow relationship for the kidney. This phenomenon of large changes in renal perfusion pressure in a kidney perfused at constant flow has been noted previously (4).

Mean blood flow to the kidney was measured using a cannulating electromagnetic flowmeter (Biotronix Laboratories) incorporated into the perfusion circuit and was recorded on an ultraviolet light recorder (Honeywell Visicorder 1508). Zero flow was recorded at the beginning of the experiment, before ligation of the descending aorta between the two renal arteries, and at the end of the experiment. Therefore, blood flow to the kidney was never interrupted during the procedure. The flowmeter was calibrated using the dog's own blood at the end of the experiment. Hind limb flow was obtained by calibration of the dial settings on the roller pump using the dog's own blood at the end of the experiment. In some experiments constancy of flow to the hind limb was verified with a second electromagnetic flow probe.

Brachial arterial pressure was recorded through a Teflon tube (8 cm long, 1 mm bore). Left atrial pressure was also recorded through a Teflon tube (12 cm long, 1 mm bore). Mean femoral perfusion pressure and mean renal perfusion pressure were recorded through polyvinyl tubes (15 cm long) connected to side branches of their respective perfusing cannulas (Fig. 1). With the glass Y-shaped tube in the descending aorta, this side branch was one side of the Y. With the stainless steel femoral cannula, this side branch was a stainless steel tube that led from immediately inside the tip of the cannula to outside the cannula. In three experiments, the right external jugular vein was cannulated with a Teflon tube (15 cm long) to record central venous pressure.

The brachial arterial pressure, femoral perfusion pressure, renal perfusion pressure, left atrial pressure, and central venous pressure were recorded from the appropriate cannulas attached to Statham P23Gb strain gauges. After amplification by a d-c amplifier (Honeywell Accudata 113) the pressures were recorded on the ultraviolet light recorder. The manometers were calibrated in steps using mercury and saline manometers. Zero pressure for the manometers measuring left atrial pressure and central venous pressure were recorded post-mortem as the levels of the tips of the cannulas free in air. The frequency response of the system recording brachial arterial pressure, obtained by the method of Hansen (10), was flat (± 5%) to better than 35 Hz. Mean pressures were obtained electrically. The electrocardiogram was recorded from leads on the forelegs and the chest wall; heart rates were counted from the electrocardiogram over periods of at least 20 seconds.

During the surgical procedures, the dogs received a slow infusion of 100 ml of dextran (6% Dextran 75 in 0.9% sodium chloride, Travenol Laboratories Inc.) for each 13 kg body weight (approximately 10% of their estimated blood volume). After the surgical procedures had been completed, a priming dose of heparin (500 units/kg, iv) was injected (heparin sodium 100 units/mg dissolved in 0.9% saline to make a concentration of 1,000 units/ml, Nutritional Biochemicals Corp.). This injection was followed with subsequent injections of heparin (1000 units, iv) every 30 minutes.

EXPERIMENTAL PROTOCOL

The experiments were performed on 19 dogs. In 4 dogs only the kidney was perfused, and in the remaining 15 dogs both the hind limb and the kidney were perfused. The small balloons in the pulmonary veins were inflated in 10 dogs, and the left atrial balloon was inflated in all dogs. In the 10 dogs in which both sets of balloons were inflated, the small balloons were always inflated first. The control periods consisted of a 2-minute period before inflation of the balloons and a 3-minute period immediately following deflation of the balloons. The experimental period was the 3-minute period during which the small balloons were inflated with 1 ml of saline. The response to the small balloons was tested three times in each dog. The interval between successive balloon inflations was approximately 3 minutes. The experimental procedures for the left atrial balloon followed similar time intervals. However, the left atrial balloon was inflated with progressively increasing volumes of saline in increments of approximately 5 ml until the change in mean left atrial pressure exceeded 25 cm H_2O. The procedure was then randomized so that approximately two tests were made at each volume. In 6 dogs, the vagus nerves were cut on the left side at the level of the upper border of the aortic arch and on the right side at the level of the azygos vein. The tests with the left atrial balloon were then repeated. Cervical vagotomy was performed in 12 dogs including the 6 dogs in which thoracic vagotomy had been performed; after the return to a steady cardiovascular state (usually about 5 minutes) the tests with the left atrial balloon were repeated.

Before each experiment was started, the ability of the preparation to respond reflexly was checked by occluding both carotid arteries. In every case there was an obvious, significant vasoconstriction in both the renal and the hind-limb vascular beds. This test was repeated at intervals throughout the experiment. No quantification of these responses was attempted, since the stimulus provided by carotid occlusion was uncontrolled.

MEASUREMENTS AND CALCULATIONS

Measurements of pressure and flow were averaged over the final minute of each control and experimental period, or, if significant Mayer waves were present, these measurements were averaged over the final 2 minutes of the period. The pressure and flow records were read using a planimeter. An estimate of the reproducibility of measurement with this instrument was obtained by making repeated readings on the same record and observing the range of deviation about the mean of the repeated readings. The systemic pressures were estimated with a reproducibility of measurement of ± 2 mm Hg. The mean venous pressures were estimated with a reproducibility of measurement of ± 1 cm H_2O. Changes in renal blood flow of 3 ml/min could be measured using the planimeter. The absolute value for flow was accurate to approximately ± 10 ml/min owing to zero drift over the whole experimental period. Therefore, all changes in renal blood flow of less than 3 ml/min were recorded as no change and all changes in pressure of less than 2 mm Hg were recorded as no change.

The raw data obtained during the control periods before and after the experimental period were averaged and compared with the raw data obtained during the experimental period using Student's *t*-test for paired data. Comparisons between groups of tests were made using Student's *t*-test for unpaired data.

For the purpose of comparison in the figures, vascular resistances in the kidney and the hind limb were calculated as the ratio of pressure to flow. Changes were expressed as the percent change in resistance from control values.

Results

In each test period the pressure perfusing the kidney and the blood flow to the hind limb remained constant despite changes in renal flow and hind-limb perfusion pressure. The mean renal perfusion pressure for all experiments was 122 ± 4.5 (SE) mm Hg and the mean hind-limb flow for all experiments was 64 ± 5.7 (SE) ml/min.

EFFECTS OF OBSTRUCTION OF THE MITRAL ORIFICE

Inflation of the large balloon in the left atrium partially blocked the mitral orifice, obstructing blood flow and raising left atrial pressure. This technique raises pressure throughout the pulmonary vascular bed, but the increase in pressure is not transmitted to the right atrium (9). In the three dogs in which central venous pressure was measured, it was 9 ± 0.7 cm H_2O; distention of the left atrium with all distending pressures, including one increase in left atrial pressure of 44 cm H_2O, did not change the central venous pressure.

The effects of distention of the large balloon were grouped according to the increase in pressure in the left atrium which occurred when the large balloon was inflated (Table 1). The partial obstruction of the mitral orifice to cause a graded increase in left atrial pressure always caused an immediate decrease in arterial blood pressure and an increase in heart rate. The effect of partial obstruction of the mitral orifice on the vascular resistance in the hind limb was opposite to its effect on renal vascular resistance. An average increase in left atrial pressure of 7 cm H_2O caused an increase in renal blood flow but had no significant effect on hind-limb pressure. Further increases in left atrial pressure of 13 cm H_2O and 18 cm H_2O caused significant increases in both renal blood flow and hind-limb perfusion pressure. Increases in left atrial pressure of 23 cm H_2O and 31 cm H_2O caused greater increases in hind-limb perfusion pressure but had no significant effect on renal blood flow. The

TABLE 1

Cardiovascular Effects of Left Atrial Distention in 19 Dogs

	Left atrial pressure (cm H_2O)		Heart rate (beats/min)		Arterial blood pressure (mm Hg)		Renal blood flow (ml/min)		Limb pressure (mm Hg)	
	C	E	C	E	C	E	C	E	C	E
N	25		23		25		25		19	
MEAN ± SE	7 ± 0.84	14 ± 0.95	162 ± 10.06	175 ± 8.90	141 ± 3.60	136 ± 4.28	149 ± 15.71	155 ± 16.09	111 ± 5.20	113 ± 5.33
d	7		13		−5		6		2	
t			3.79		2.98		2.82		0.75	
P			< 0.001		< 0.005		< 0.005		NS	
N	20		17		20		20		20	
MEAN ± SE	6 ± 0.87	19 ± 0.98	169 ± 13.39	190 ± 11.21	136 ± 4.63	128 ± 5.95	183 ± 19.82	192 ± 19.58	112 ± 4.88	120 ± 6.53
d	13		21		−8		9		8	
t			4.65		4.32		2.96		2.06	
P			< 0.001		< 0.001		< 0.005		< 0.05	
N	31		27		31		31		24	
MEAN ± SE	6 ± 0.83	24 ± 0.88	184 ± 8.81	204 ± 6.55	140 ± 4.29	130 ± 5.89	151 ± 12.81	156 ± 13.11	119 ± 5.36	132 ± 8.11
d	18		20		−10		5		13	
t			5.89		4.27		2.15		3.18	
P			< 0.001		< 0.005		< 0.05		< 0.005	
N	19		17		19		19		14	
MEAN ± SE	8 ± 0.82	31 ± 0.99	205 ± 8.01	220 ± 6.48	132 ± 4.65	117 ± 5.09	116 ± 12.60	119 ± 14.26	116 ± 8.70	139 ± 17.22
d	23		15		−15		3		23	
t			5.08		7.12		0.79		2.24	
P			< 0.001		< 0.001		NS		< 0.05	
N	18		17		18		18		17	
MEAN ± SE	6 ± 0.75	37 ± 1.58	197 ± 10.43	211 ± 8.59	138 ± 5.02	122 ± 5.39	177 ± 19.05	173 ± 19.03	119 ± 5.26	144 ± 9.57
d	31		14		−16		−4		25	
t			2.87		5.81		1.70		4.30	
P			< 0.01		< 0.001		< 0.10		< 0.001	

Results are grouped according to the changes in left atrial pressure (1–10, 11–15, 16–20, 21–25, and >26 cm H_2O). C = control period, E = experimental period, and *t* = statistical data derived from Student's *t*-test.

differences between the responses to mitral obstruction observed in the hind limb and the kidney are emphasized in Figure 2 which shows the relationship between the increase in left atrial pressure and the changes in hind-limb and renal vascular resistance.

EFFECTS OF MITRAL OBSTRUCTION AFTER VAGOTOMY

In the 12 dogs in which vagotomy was performed, increases in left atrial pressure before vagotomy caused effects similar to those reported in Table 1 for all 19 dogs.

The effects of distention of the left atrium in six dogs in which thoracic vagotomy was performed are given in Table 2. Section of the vagus nerves at the level of the upper border of the aorta on the left side and the azygos vein on the right side did not cause any significant change in the control level of heart rate or arterial blood pressure. Increases in left atrial pressure of less than 15 cm H_2O caused changes in heart rate, arterial blood pressure, and hind-limb pressure comparable with those observed before vagotomy but did not cause any increase in renal blood flow. Further increases in left atrial pressure not only caused greater changes in arterial blood pressure and hind-limb pressure than they did before thoracic vagotomy but also

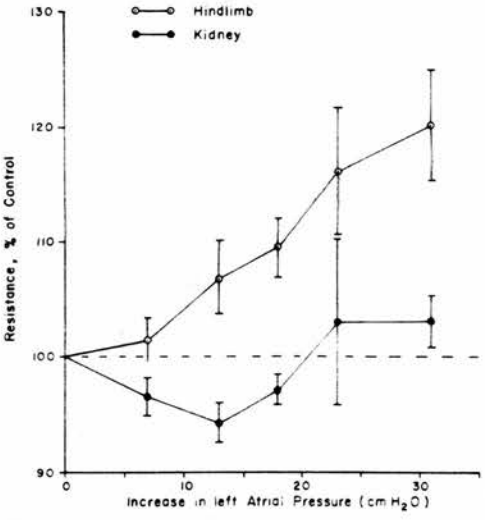

FIGURE 2

Changes in vascular resistance in the hind limb and kidney during distention of the left atrium. Abscissa shows increases in left atrial pressure. Vascular resistance is expressed as a percent of control values (control = 100%). Averages ± SE from 19 dogs are given.

TABLE 2

Cardiovascular Effects of Left Atrial Distention after Thoracic Vagotomy in Six Dogs

	Left atrial pressure (cm H_2O)		Heart rate (beats/min)		Arterial blood pressure (mm Hg)		Renal blood flow (ml/min)		Limb pressure (mm Hg)	
	C	E	C	E	C	E	C	E	C	E
N	16		14		16		16		16	
MEAN ± SE	7±0.70	17±1.00	128±4.14	145± 6.04	110±5.04	102±4.97	188±11.23	184±11.33	113± 5.14	124± 6.45
d	10		17		−8		−4		11	
t			3.69		4.59		1.49		3.85	
P			< 0.005		< 0.001		< 0.10		< 0.005	
N	9		7		9		9		9	
MEAN ± SE	7±0.78	28±0.94	139±8.00	162±10.94	99±5.63	76±8.36	161±13.02	148±13.89	108± 7.84	131±14.91
d	21		23		−23		−13		23	
t			4.99		4.58		4.75		2.63	
P			< 0.005		< 0.005		< 0.005		< 0.05	
N	5		5		5		5		5	
MEAN ± SE	7±1.21	38±0.73	130±5.88	139±12.06	105±8.06	57±7.71	172±21.12	130±15.94	116±11.64	149±23.16
d	31		9		−52		−42		33	
t			0.77		3.49		2.93		1.64	
P			NS		< 0.05		< 0.05		< 0.10	

Results are grouped according to changes in left atrial pressure (1-15, 16-25, >25 cm H_2O). C = control, E = experimental, and *t* = statistical data derived from Student's *t*-test.

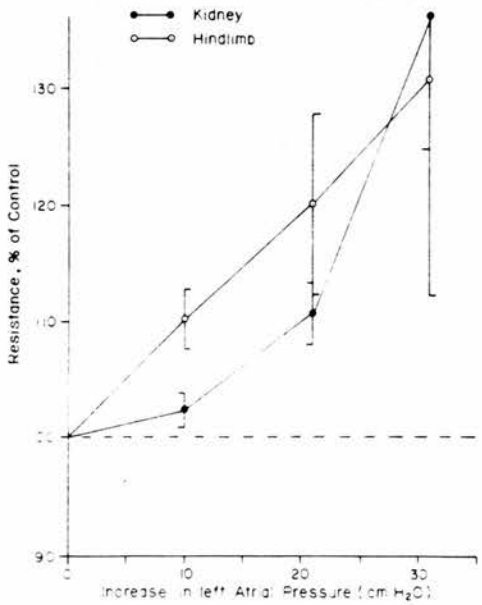

FIGURE 3

Changes in vascular resistance in the perfused hind limb and kidney during graded distention of the left atrium in six dogs. Results are shown after section of the right vagus nerve at the level of the azygos vein and the left vagus nerve at the upper border of the aorta. Vascular resistance is expressed as a percent of control values (control = 100%). Values are means ± SE.

caused significant decreases in renal blood flow. Figure 3 shows the relationship between the increase in left atrial pressure and the changes in hind-limb and renal resistances after thoracic vagotomy.

The effects of distention of the left atrium in 12 dogs in which cervical vagotomy was performed are given in Table 3. An increase in left atrial pressure after cervical vagotomy did not cause any significant changes in heart rate, which was already high. After cervical vagotomy left atrial distention caused somewhat variable increases in hind-limb perfusion pressure, but renal blood flow was always decreased. The decreases in renal blood flow were not significantly different from those observed after thoracic vagotomy.

EFFECTS OF DISTENTION OF THE PULMONARY VEIN-LEFT ATRIAL JUNCTIONS

Three pulmonary vein-left atrial junctions were distended by small balloons a total of 26 times in ten dogs. In the steady state, 3 minutes after distention of the pulmonary vein-left atrial junctions, there was no effect on left atrial pressure (8.3 ± 1.1 cm H_2O), on brachial arterial pressure (154 ± 3.9 mm Hg), or on hind-limb perfusion pressure (control value 115 ± 3.9 mm Hg, experimental value during pulmonary vein distention 116 ± 3.7 mm Hg). However, distention of the pulmonary vein-left atrial junctions did cause a significant ($P < 0.001$) increase in heart rate and a significant ($P < 0.01$) increase in renal blood flow. Heart rate increased from a control value of 176 ± 8 beats/min to an experimental value of 186 ± 8 beats/min, and renal blood flow increased from a control value of 139 ± 7.8 ml/min to an experimental value of 142 ± 7.7 ml/min. This average increase in renal blood flow represented a 2.2% decrease in renal vascular resistance. The high statistical significance of this change despite its small magnitude can be accounted for by the use of Student's *t*-test for paired data and the fact that there was a decrease in renal blood flow in only 3 of the 26 tests. The high standard errors are due to the wide variation in renal blood flow between dogs.

Parts of the steady-state records of a test in which the pulmonary vein-left atrial junctions were distended are shown in Figure 4. In this test distention of the pulmonary vein-left atrial junctions caused renal blood flow to increase from a control value of 179 ml/min to an experimental value of 188 ml/min, and, following removal of the distention, renal blood flow returned to a control value of 178 ml/min. Measurements of the pressure records using a planimeter over 1-minute periods indicated that during distention there was no change in limb pressure (126 mm Hg), brachial arterial pressure (140 mm Hg), or mean left atrial pressure (4 cm H_2O). Renal perfusion pressure was constant at 140 mm Hg. Heart rate during the experimental period was 4 beats/min faster than the average heart rate during the control periods. Thus the only effects of distention of three pulmonary vein-left atrial junctions were an increase in heart rate and a dilatation in the kidney.

Discussion

A partial obstruction of the mitral orifice that raised left atrial pressure less than 20 cm H_2O caused hypotension, tachycardia, and vasodilatation in the kidney and vasoconstriction in the hind limb. A partial obstruction of the orifice that raised left atrial pressure more than 20 cm H_2O caused a greater hypotension, tachycardia, and hind-limb vasoconstriction but was not accompanied by dilatation in the kidney. After thoracic vagotomy,

TABLE 3

Cardiovascular Effects of Left Atrial Distention after Cervical Vagotomy in 12 Dogs

	Left atrial pressure (cm H_2O)		Heart rate (beats/min)		Arterial blood pressure (mm Hg)		Renal blood flow (ml/min)		Limb pressure (mm Hg)	
	C	E	C	E	C	E	C	E	C	E
N	27		18		27		27		27	
Mean ± SE	7 ± 0.56	18 ± 0.88	193 ± 7.01	195 ± 7.79	118 ± 7.58	99 ± 9.18	129 ± 9.33	119 ± 9.02	115 ± 8.91	119 ± 9.82
d	11		2		-19		-10		4	
t			0.94		5.69		4.74		2.35	
P			NS		<0.0005		<0.0005		<0.05	
N	17		13		17		17		16	
Mean ± SE	10 ± 1.51	30 ± 1.55	182 ± 10.35	183 ± 11.10	126 ± 7.91	105 ± 10.80	134 ± 12.23	117 ± 10.83	126 ± 9.02	132 ± 10.00
d	20		1		21		17		6	
t			0.09		4.04		4.13		1.96	
P			NS		<0.0005		<0.0005		<0.05	
N	9		6		9		9		9	
Mean ± SE	7 ± 1.15	36 ± 1.72	200 ± 16.35	194 ± 20.72	128 ± 9.19	82 ± 14.46	106 ± 14.93	76 ± 12.24	141 ± 13.23	174 ± 19.38
d	29		-6		-46		-30		33	
t			0.89		6.28		4.40		3.92	
P			NS		<0.0005		<0.005		<0.005	

Results are grouped according to changes in left atrial pressure (1–15, 15–25, >26 cm H_2O). C = control, E = experimental, and *t* = statistical data derived from Student's *t*-test.

partial obstruction of the mitral orifice caused vasoconstriction in both the kidney and the hind limb, and this response was little altered by subsequent cervical vagotomy. The observed responses of the hind limb and the kidney must be attributed to a reflex secondary to a change in receptor input brought about by partial obstruction of the mitral orifice.

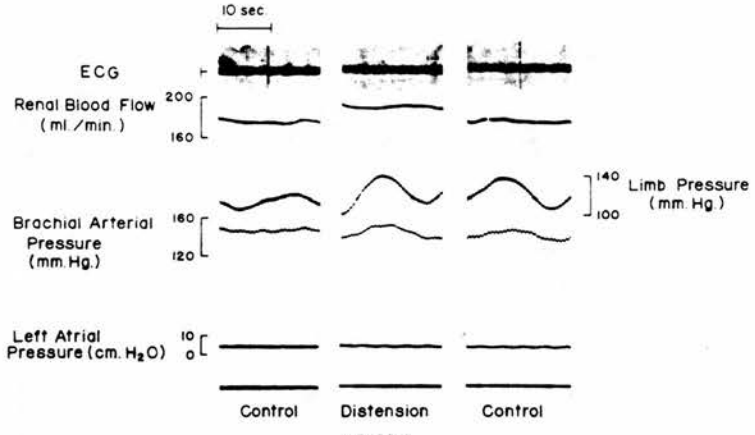

FIGURE 4

Parts of the record of one experiment in which small balloons were distended in each of three pulmonary veins. **Left:** *control values.* **Center:** *Values during pulmonary vein distention.* **Right:** *Control values 3 minutes after removal of the distention.*

The stimulus provided by acute mitral obstruction is not limited to the left atrium, because pressures are raised in the pulmonary artery (9) and presumably throughout the pulmonary vascular bed. Right atrial receptors (11, 12) are probably unaffected because central venous pressure does not change. Stimulation of pulmonary arterial baroreceptors (13) causes a fall in systemic arterial blood pressure, but there is no information on the relative effects of stimulation of these receptors on the hind limb and the kidney. Lung inflation, which stimulates slowly adapting pulmonary stretch receptors causes hypotension with pronounced dilatation in the hind limb, skin, and muscle (14). However, Marshall and Widdicombe (15) have demonstrated that distention of a balloon in the left atrium which raises left atrial pressure 20–40 cm H_2O causes only a small increase in the discharge rate of these receptors at any lung volume. Pulmonary congestion has been shown to stimulate type J pulmonary receptors (16). Stimulation of type J receptors probably causes bradycardia and hypotension (17), but again there is no information regarding relative effects on the renal and hind-limb vascular resistance. Therefore, there is no experimental evidence to indicate that stimulation of receptors by pulmonary congestion is responsible for the observed dilatation in the kidney when there is constriction in the hind limb.

A rise in left atrial pressure induced by partial obstruction of the mitral orifice is associated with an increase in afferent discharge from the left atrial receptors (6). Section of the right vagus nerve above the azygos vein and section of the left vagus nerve at the upper border of the aorta (thoracic vagotomy in the present experiments) cut the afferent fibers from the lungs and probably divide most of the afferent fibers from receptors in the left side of the left atrium (18). However, these sections leave intact most of the efferent vagal fibers to the heart (7) and most of the afferent fibers from the pulmonary arterial baroreceptors, aortic baroreceptors, and aortic chemoreceptors (18). Cutting the vagus nerves at these levels reduces the diuretic response to left atrial distention (19) and almost completely prevents the increase in heart rate caused by distention of the left pulmonary vein-left atrial junctions (7). Thus, the alteration of the renal vascular response to atrial distention by thoracic vagotomy is probably due to interruption of afferent fibers from the left atrium. Afferent fibers from the lungs might also have contributed to the response, although this possibility seems unlikely for reasons already discussed.

In comparing the vascular response in the kidney with that in the hind limb during left atrial distention, it is necessary to consider the contributions of both the increased stimulus to the cardiac and intrapulmonary receptors and the decreased stimulus to the arterial baroreceptors. Öberg and White (2) have shown in the cat that section of the carotid sinus nerves causes greater vasoconstriction in the hind limb than it does in the kidney; however, later section of the cardiac vagal nerves causes greater constriction in the kidney than it does in the hind limb. More recently, Öberg and Thören (20) have suggested that stimulation of nonmedullated afferent fibers which presumably arise from the ventricles causes marked slowing of the heart and dilatation in the kidney and muscle vessels. The degree of dilatation reported does not appear to be greater in the kidney than it is in muscle vessels. Such ventricular receptors might have been stimulated by mitral obstruction because peak right ventricular pressure is increased by this technique. However, stimulation of such receptors probably did not cause dilatation in the kidney without causing it in the hind limb. There is indirect evidence in rabbits (1, 21) and dogs (4) that the main influence of cardiopulmonary receptors is on the renal circulation, whereas the main influence of the arterial baroreceptors is on the circulation to the extremities. Therefore, the net result of an increased stimulus to cardiac and intrapulmonary receptors and a decreased stimulus to the aortic baroreceptors might then be renal dilatation and constriction in the hind limb, as observed in the present experiments. The fact that no constriction was observed in the kidney before vagotomy (Fig. 2.) when there was a significant increase in hind-limb resistance might be an indication of the magnitude of the effects of stimulation of low-pressure receptors on the renal circulation.

In an attempt to localize more precisely a receptor group within the intrathoracic circulation that was at least partially responsible for the dilatation in the kidney, three pulmonary vein-left atrial junctions were distended by small balloons. This technique does not obstruct blood flow through the left atrium (7), and it provides a marked stimulus to the receptors situated in the subendocardium at the pulmonary vein-left atrial junctions (8). Previously, stimulation of this area has been shown to cause an increase in heart rate without any effects on myocardial contractility or hind-limb resistance in the steady state (7, 22, 23). More recently, it has been demonstrated (5) that distention of the pul-

monary vein-left atrial junctions by small balloons causes increases in cardiac and decreases in renal sympathetic nerve activity. Also, Karim et al. (5) have found no change in sympathetic activity to the hind limb during pulmonary vein distention, and, in the present experiments, we were unable to demonstrate any dilatation in the hind limb. Thus, the effect of pulmonary vein distention is a dilatation localized to the kidney and not a generalized dilatation that merely affects the renal vascular bed somewhat more than other vascular territories.

The results reported in this paper demonstrate that the decrease in renal sympathetic nerve activity previously observed during distention of the pulmonary vein-left atrial junction (5) results in a decrease in renal vascular resistance. Although the observed changes in total renal vascular resistance were small, there is no evidence that this change was the only effect on the kidney of the decreased sympathetic activity. More profound effects on renal function might be expected if there was also a redistribution of blood flow between the renal medulla and the cortex (24). Left atrial distention is also associated with a diuretic response from the kidney (9). The diuresis might be mainly the result of inhibition of the release of antidiuretic hormone from the neurohypophysis (25), but the time course and the characteristics of solute excretion during the diuresis suggest that there might also be a hemodynamic component (26, 27). It is possible that the hemodynamic component of the diuretic response is provided by the decrease in renal vascular resistance secondary to an increased stimulus to left atrial receptors.

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J. Physiol. (1977), **273**, *pp.* 57–67
With 3 text-figures
Printed in Great Britain

THE RESPONSE TO DISTENSION OF THE PULMONARY VEIN–LEFT ATRIAL JUNCTIONS IN ANAESTHETIZED DOGS AFTER SECTION OF THE ROSTRAL MEDULLA

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(*Received 22 November 1976*)

SUMMARY

1. Distension of the pulmonary vein–left atrial junctions caused an increase in heart rate and a transient decrease in mean arterial pressure.

2. Section of the brain stem at the level of the inferior cerebellar peduncle (rostral medulla) caused a decrease in mean arterial pressure.

3. Section of the rostral medulla had no effect on either the magnitude or the time course of the reflex response to pulmonary vein distension.

4. Administration of propranolol after section in the rostral medulla reduced the reflex increase in heart rate in response to pulmonary vein distension by an amount similar to that previously described in intact and decerebrate animals.

5. Bilateral cervical vagotomy prevented the reflex response to pulmonary vein distension.

INTRODUCTION

Distension of the pulmonary vein–left atrial junctions by means of small balloons has been shown to cause a reflex increase in heart rate (Ledsome & Linden, 1964). Pulmonary vein distension is an effective stimulus to left atrial receptors (Linden, 1972), the afferent fibres from which travel in the vagus nerves and enter the medulla. The further course of the reflex pathway is unknown. However, the magnitude and time course of the reflex response is not affected by mid-collicular decerebration (Albrook, Bennion & Ledsome, 1972). Recently neurones, the activity of which varied during atrial pulsation or blood volume infusion (presumed to stimulate atrial receptors) have been described in the nucleus tractus solitarius (NTS) and in the underlying reticular formation of the para-hypoglossal region (Baertschi, Munzner, Ward, Johnson & Gann, 1975).

Also degeneration studies have indicated termination of vagal afferent fibres in the intermediate and caudal regions of the NTS (Cottle, 1964).

To further study the central nervous pathway of the reflex response to pulmonary veins distension it was necessary to visualize the dorsal aspect of the medulla in the dog. This entailed either piercing the vermis of the cerebellum or deflecting the cerebellum rostrally. The medial reticular nuclei receive projections from the cerebellar and vestibular nuclei (Korner, 1971) and it has been demonstrated that in the cat cerebellectomy and section of the brain stem in the low pons both alter the reflex response to carotid occlusion (Reis & Cuenod, 1965). Also it has been shown that in particular the fastigial nucleus projects to the medulla in the region of the parahypoglossal nucleus (Brodal & Gogstad, 1957; Miura, Kawamamura & Reis, 1969) and that stimulation in the fastigial nucleus inhibits reflex vagal bradycardia (Achari & Downman, 1970). The present experiments were designed to demonstrate whether section of the brain stem at the level of the inferior cerebellar peduncle, which effectively removes any cerebellar connexions with the medulla, influences the reflex response to pulmonary vein distension in the anaesthetized dog.

METHODS

Mongrel dogs of both sexes and weighing 10–14 kg were anaesthetized 0·5 h after the s.c. injection of morphine sulphate, 0·5 mg/kg. Alpha chloralose (British Drug Houses; 1 % (w/v) solution in 0·9 % sodium chloride) was infused in a dose of 10 ml./kg via a cannula inserted under local anaesthesia (Winthrop Laboratories: carbocaine, 1 %) into the left lateral saphenous vein. A steady state of light anaesthesia was maintained throughout the course of the experiment by the continuous infusion of 1 ml./min of a 0·5 % solution of chloralose. The constant infusion was started approximately 15 min after the initial dose of anaesthetic, through a cannula in the right external jugular vein.

After the induction of anaesthesia a thermistor probe was placed in the oesophagus and the animals' temperature was maintained at 37 °C (± 2 °C) by a heated table. The trachea was cannulated and the carotid sheath on both sides dissected free from surrounding structures. Soft cord was placed around each freed vagosympathetic nerve trunk to expedite later identification.

The right femoral artery was cannulated with a 15 cm piece of Teflon tubing (1 mm bore) and femoral arterial pressure measured using a strain gauge transducer (Statham Inst. Co. P23 Gb). After amplification by a DC amplifier (Honeywell, Accudata 113) pressure was recorded on a direct writing ultra-violet light recorder (Honeywell, 1508). The frequency response of this system for measuring arterial pressure, as determined by the method of Hansen (1949) was flat ± 5 % to better than 35 Hz. Mean pressure was obtained electrically.

Samples of arterial blood were taken at intervals throughout the experiment and P_{CO_2}, P_{O_2} and pH measured using appropriate electrodes and amplifiers (Instrumentation Laboratories, Model no. 127). If necessary to keep the P_{CO_2} and pH within the normal ranges of 35–40 mmHg and 7·3–7·4 pH units respectively, sodium bircar-

bonate (1 M) was injected I.V. or adjustments to the stroke volume of the respiratory pump were made.

A two-lead e.c.g. was attached to the chest wall and after preamplification (Grass Inst. P-15) was displayed simultaneously on both the ultraviolet recorder and a dual-beam oscilloscope (Tektronix, RM 565). Heart rate was recorded by means of a cardiotachometer triggered by the R-wave of the e.c.g. All heart rates reported in the experimental results were counted directly from the e.c.g. record over at least 0·5 min.

The animal was placed on its right side, and a left lateral thoracotomy performed at the fifth intercostal space. A Harvard respirator was attached to the tracheal cannula with a stroke volume of approximately 50 ml./3 kg body wt., at a rate of 18 breaths/min. Oxygen (1 l./min) was added to the inspired air to ensure adequate oxygenation. As the chest was opened a resistance to expiration of 3 cm H_2O was supplied. Dextran (Baxter Laboratories; travenol, 6 % Dextran in normal saline) was infused in a volume approximately equal to 10 % of the estimated blood volume. After deflecting the lobes of the left lung dorsally so as to expose the pulmonary veins, small balloons (approximately 3 mm in length) were inserted into each of three pulmonary veins after the manner described by Ledsome & Linden (1964). The left lung was then tied off at the root with stout cord.

The animal was placed in a prone position with the head fixed in a stereotaxic frame (La Précision Cinématique, Paris). The position of the head was standardized despite wide variations in head size by placing the inter-aural zero at 40 mm on the horizontal frame and fixing the angle of the head with the mandibular clamp so that the lateral canthus of the eye was in the same horizontal plane as the ear bars. The neck muscles were divided in the mid line and their rostral insertions into the nuchal crest divided. A rectangular portion of the supraoccipital bone was then removed using a small electric drill and rongeurs. The dura overlying the cerebellum and caudal medulla was trimmed away and the size of the hole enlarged by continuing the incision of the dura over the foramen magnum.

Section of the brain stem was accomplished by means of a bank of stainless-steel electrodes constructed in a manner previously described (Albrook *et al.* 1972) for the decerebration of dogs. Two modifications were made to allow section of the rostral medulla; because of the narrower opening only five electrodes were used, and all were of equal length (100 mm). The electrodes were lowered in the mid line through the vermis of the cerebellum at approximately a 55° angle to the horizontal. Lowering them at this angle about midway between the foramen magnum and the rostral edge of the opening resulted in a section of the brain stem through or just caudal to the inferior cerebellar peduncle. Coagulation with a high frequency coagulator (Wyss coagulator, J. Monti, Geneva, current 50 μA for 15 sec) was done in five 2 mm steps starting from the ventral surface of the brain stem. Depending on the size of the dog and the width of the opening, the electrodes were again raised, moved first to the extreme right-hand edge, relowered and the coagulation repeated. This process was repeated with the electrodes at the extreme left-hand edge. This was found to be necessary to ensure as complete a section as possible. Each experiment was assessed visually for level and completeness of section following exsanguination and removal of the cerebellum. A composite diagram of the sections obtained by this method in eight of the dogs reported in this series is shown in Fig. 1. In some experiments a small portion (1–2 mm) of the extreme lateral portions of the inferior cerebellar peduncles was not obviously damaged.

Experimental protocol

Upon completion of the surgical preparation and the opening of the dura over the lower brain stem the animal was allowed to recover for 10 min. Temperature, level of anaesthesia, blood gases and the general condition of the animal as reflected by heart rate and blood pressure were observed for normal or steady-state conditions before experimental procedures were begun. Testing for the appearance of the response to pulmonary vein distension was always done in the same manner. Following

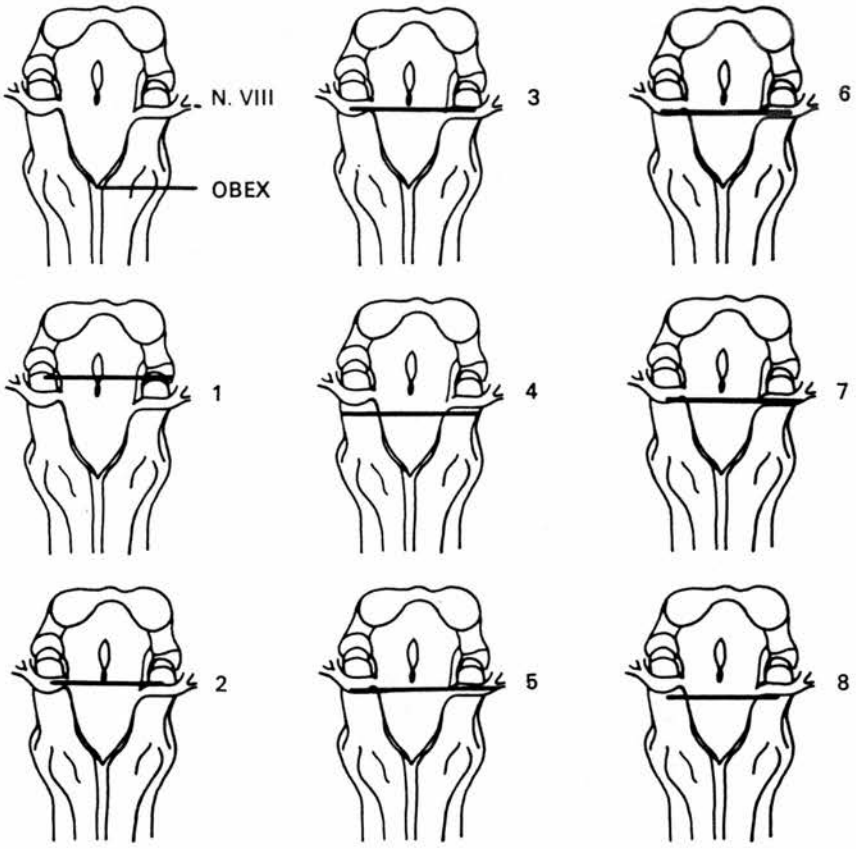

Fig. 1. Diagram of dorsal view of canine brain stem showing the position of eight transections made. The horizontal lines numbered 1–8 indicate the extent of the transections laterally. There was always complete transection in the dorso-ventral plane along these lines.

a control period, the pulmonary balloons were inflated by the injection of 0·5 ml. (8–9 kg dogs) to 1·0 ml (10–14 kg dogs) of saline; after 2 min a record was made for 1 min during the period of inflation and the balloons then deflated. After a 2 min recovery period another 1 min record was taken and the changes in heart rate and blood pressure during inflation calculated by comparison with average values before

and after inflation. Immediate changes on balloon inflation and deflation were measured over 10 sec periods, 5–15 sec and 15–25 sec after the inflation or deflation.

After completion of three such control trials the section of the lower brain stem was performed as described and the reflex response again tested. The nervous pathways involved were studied by the administration of I.V. propranolol, 0·5 mg/kg (Ayerst Laboratories, AY 64043). The β-receptor blockade resulting from this dose of propranolol was judged to be adequate if it blocked > 90% of the maximal increase in heart rate caused by the rapid intravenous injection of isoprenaline (0·5 μg/kg, K and K Laboratories: isoprenaline salt sulphate). If insufficient blocking was observed, the dose of propranolol was repeated. Testing for the response to pulmonary vein–left atrial distension was done following β-blockade and finally following cervical vagotomy. The significance of the observed changes in heart rate and blood pressure was tested using a Student's *t*-test for paired data.

RESULTS

Effects of distension of the pulmonary vein–left atrial junctions

Distension of the pulmonary vein–left atrial junctions in the control state by inflation of the pulmonary vein balloons caused a mean increase in heart rate of 19 beats/min (S.E. of mean $\pm$ 2·4; range 1–65 beats/min) in thirty trials in ten dogs. The increase in heart rate occurred in all dogs from all levels of control heart rate ranging from 54 to 189 beats/min. The increase in heart rate was not accompanied by any significant changes in femoral arterial pressure, the mean change in the thirty trials being + 0·8 mmHg (S.E. of mean $\pm$ 1·2; range − 9 to + 14 mmHg).

The time course of the changes was of interest, as some workers have questioned the validity of accurately describing the response to pulmonary vein distension solely on the basis of steady-state changes (Paintal, 1973). Heart rate increased rapidly in the first 20 sec of balloon inflation, and in only two of the thirty trials was the heart rate unchanged in this period. Examination of the changes in arterial blood pressure during this same time period revealed a somewhat different pattern of response. Whereas the changes in heart rate were large the changes in arterial pressure were relatively small and occurred in only about half the dogs. Nevertheless in these animals the pattern of the changes was reproducible. Within 10 sec of balloon inflation there was a fall in arterial pressure which reached its nadir between 10 and 20 sec after inflation and then gradually increased, so that arterial pressure returned to its steady-state value within 20–50 sec. The average changes over the first 25 sec of inflation and in the steady state 2–3 min after inflation are shown in Fig. 2. A record of this type of response has been published previously (Carswell, Hainsworth & Ledsome, 1970).

A similar examination of the time course of the reverse events occurring at balloon deflation was also made. The major difference observed was

that the decrease in heart rate initiated on removal of the stimulus was slower than the increase which had previously occurred at application of the stimulus. There were no significant transient changes in mean arterial pressure on balloon deflation corresponding in any way to the transient fall in arterial pressure observed upon inflation.

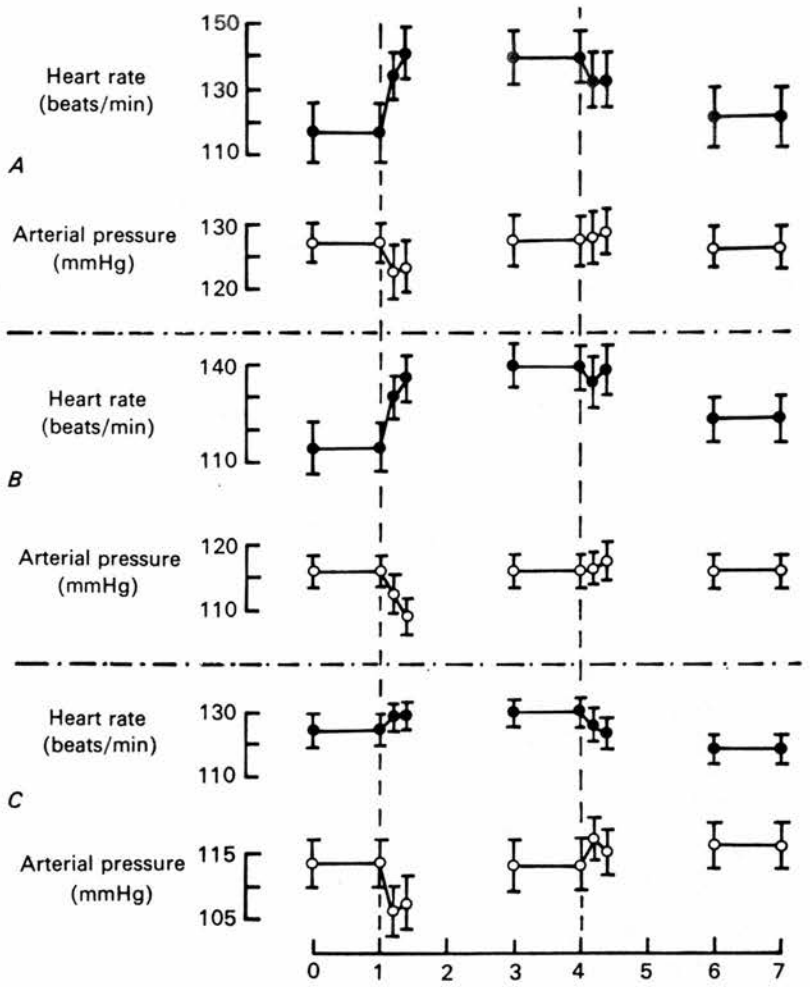

Fig. 2. Heart rate and arterial pressure before, during and after distension of the pulmonary vein–left atrial junctions. Panel *A*, ten intact animals; panel *B*, ten animals after section of brain stem in the rostral medulla; panel *C*, eight animals as in B but after propranolol. Each point represents the mean (± S.E. of mean) of the average response (three tests) in each animal.

Effects of section of the brain stem in the rostral medulla

The separation of structures rostral to the inferior cerebellar peduncle by high-frequency coagulation of the rostral medulla–pons junction (Fig. 1) brought about a decrease in both heart rate and arterial blood pressure in ten dogs. The heart rate decreased from a mean of 120 beats/min (S.E. of mean $\pm$ 16; range 60–186) immediately before section to a mean of 105 beats/min (S.E. of mean $\pm$ 15; range 52–186) approximately 5 min after the section was completed. The heart rate after section was not significantly different from the control heart rate ($0{\cdot}1 < P < 0{\cdot}2$). Blood pressure followed a parallel course decreasing from a control value of 129 mmHg (S.E. of mean $\pm$ 7·2; range 109–174) to a post-section value of 111 mmHg (S.E. of mean $\pm$ 4·2; range 96–144) which was a significant decrease ($P < 0{\cdot}025$). The decrease in heart rate was sustained during the experimental period in six dogs; in the other four dogs there was a slow gradual increase in heart rate throughout the experiment. Blood pressure also remained stable following section of the rostral medulla.

Effects of distending the pulmonary vein–left atrial junctions after section of the rostral medulla

Inflation of the pulmonary vein balloons after the condition of the animals had stabilized at the levels described above caused an increase in heart rate without significant changes in blood pressure, a response indistinguishable from that observed before section. In thirty trials in ten dogs the mean increase in heart rate was 20 beats/min (S.E. of mean $\pm$ 2·7; range 2–65). The mean change in arterial blood pressure during these trials was $-0{\cdot}4$ mmHg (S.E. of mean $\pm$ 1·5, range -26 to $+10$). Not only the magnitude of the final steady-state response but also the immediate changes observed on pulmonary vein distension were unaltered by the section (Fig. 2). Both the time course and the direction of the changes in heart rate and blood pressure at 10 sec, 20 sec and 2 min after balloon inflation and deflation were similar to those occurring in the intact animal. Also the scatter of the steady-state changes in heart rate and arterial pressure was similar (Fig. 3).

Effects of propranolol and cervical vagotomy on the reflex response

Previous investigations have demonstrated that the increase in heart rate initiated by distension of the pulmonary vein–left atrial junctions is a reflex mediated by afferent fibres travelling in the vagus nerves and efferent fibres travelling mainly in the cardiac sympathetic nerves (Ledsome & Linden, 1964). Characterizing the response on the basis of its

reduction or abolition by the action of propranolol and its subsequent total disappearance after cervical vagotomy can be considered an effective means of identifying the components of the reflex. In the present series of experiments, eight of the ten dogs submitted to sectioning of the brain stem were treated with propranolol (0·5 mg/kg). In twenty-three trials of pulmonary vein distension in these eight dogs the mean increase in heart rate was reduced to 8 beats/min (S.E. of mean ±1·5; range 1–22). The scatter of the results is shown in Fig. 3 and the time course of the response

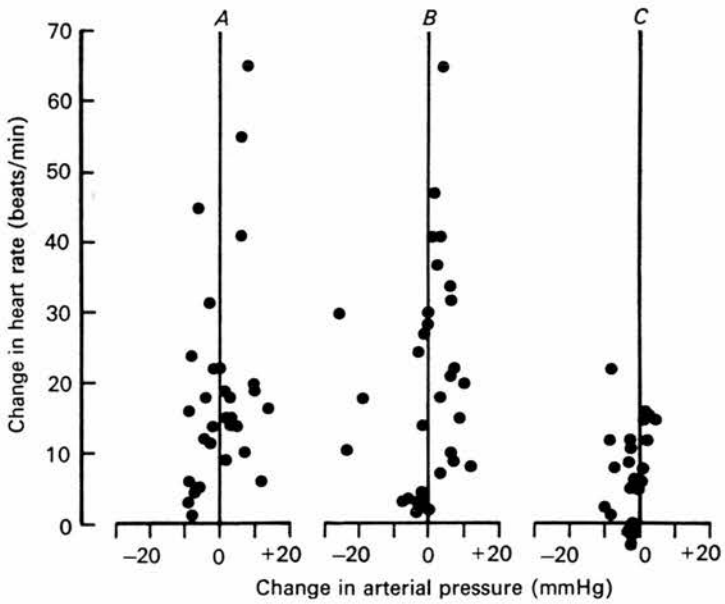

Fig. 3. Changes in heart rate and mean arterial pressure in the steady state (3 min after distension) in response to distension of the pulmonary vein–left artrial junctions. *A*, intact animals. *B*, after section in the rostral medulla. *C*, as in *B* after propranolol (0·5 mg/kg).

is plotted in Fig. 2. The results were similar to those reported previously in decerebrate dogs (Albrook *et al.* 1972). It may be noted that the small changes in heart rate which occurred on inflation and deflation of the pulmonary vein balloons reached their maximum values more rapidly than in the absence of propranolol. Average blood pressure changes were small (−2·0 mmHg; S.E. of mean ± 0·9; range −10 to +5) and the transient changes in blood pressure remained unaffected by propranolol as previously reported (Carswell *et al.* 1970).

In all cases, severing both vago-sympathetic trunks in the cervical region effectively abolished any changes in heart rate upon balloon inflation.

In twenty-five trials in nine dogs there was no change in heart rate on pulmonary vein distension (range -3 to $+3$ beats/min) and the mean blood pressure change was $-3{\cdot}2$ mmHg (S.E. of mean $\pm 1{\cdot}5$, range -21 to $+10$). There were no transient changes in heart rate or blood pressure associated with pulmonary vein distension after vagotomy. The somewhat labile nature of the arterial pressure in these animals after administration of propranolol and vagotomy is indicated by the large range of changes in arterial pressure.

DISCUSSION

There is increasing evidence for supramedullary modulation of baroreceptor reflex transmission (Kirchheim, 1976). The earlier work of Reis & Cuenod (1965) in the anaesthetized cat suggested that sections of the pons led to loss of the reflex response to carotid occlusion. However, more recently, using conscious rabbits, Korner, Shaw, West & Oliver (1972) have demonstrated that pontine section alters the balance between sympathetic and vagal effector mechanisms in the baroreceptor control of the heart rate but does not reduce the average gain of the reflex or the pressure-dependent range of the heart-rate response. The pontine animals in the latter series retained their cerebellar connections with the medulla.

Section of the brain stem has been known for some time to be associated with a decrease in heart rate (Uther, Hunyor, Shaw & Korner, 1970; Glasser, 1962). The slowing which occurs in pontine animals has been interpreted as due to interruption of a supracerebellar pathway, which in the intact animal tonically inhibits vagal motoneurones not receiving baroreceptor projections (Korner *et al.* 1972). Evidence of removal of tonic inhibition of vagal tone by decerebration in the anaesthetized dog has been previously described (Albrook *et al.* 1972). A similar decrease in heart rate and mean arterial pressure was observed in the present series after section through the rostral medulla. These results confirm that the cerebellum is not essential to the increase in vagal tone which follows brain stem section. However, caution should be used in comparing effects in so many different species.

The reflex responses to distension of the pulmonary vein–left atrial junctions in the experiments described were similar in both time course and magnitude to those that have been described previously (Ledsome & Linden, 1964; Burkhart & Ledsome, 1974). It is apparent from the results (Figs. 2, 3) that neither the magnitude nor the time course of the response was altered by sections of the rostral medulla. It is extremely unlikely that further section of the small portions of the lateral regions of the inferior cerebellar peduncle remaining in some experiments would have significantly altered these results. That the characteristics of the reflex

response were similar to those seen in decerebrate animals (Albrook *et al.* 1972; Burkhart & Ledsome, 1974) was demonstrated by adminstration of propranolol and by bilateral cervical vagotomy. Propranolol reduced the increase in heart rate in response to pulmonary vein distension, but did not completely prevent the response as has been previously described in the decerebrate animal (Albrook *et al.* 1972; Burkhart & Ledsome, 1974). However, the transient fall in arterial pressure which sometimes accompanied pulmonary vein distension was not affected by propranolol. The increase in heart rate which occurred during pulmonary vein distension after giving propranolol could be at least partly accounted for as secondary to the decrease in mean arterial pressure. However, an efferent vagal component to the reflex increase in heart rate caused by pulmonary vein distension has been demonstrated in dogs after high spinal section (Burkhart & Ledsome, 1974). Vagotomy prevented both the increase in heart rate and the transient changes in arterial pressure which accompanied left atrial distension and confirmed the reflex nature of the changes.

Section of the rostral medulla had no demonstrable effects upon either the time course or the magnitude of the response to pulmonary vein distension. It is therefore unlikely that damage to the vermis of the cerebellum which occurs when the dorsal surface of the medulla is exposed will influence the reflex response to pulmonary vein distension. The fact that the reflex response was unchanged by brain stem section should not necessarily be interpreted to imply that there can be no supramedullary modulation of the reflex response.

The skilled technical assistance of Mrs Leilani Funnell during these experiments is gratefully acknowledged. The work was supported by the Medical Research Council of Canada and the British Columbia Heart Foundation.

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J. Physiol. (1977), **273**, *pp.* 69–81
With 2 plates and 3 text-figures
Printed in Great Britain

EFFECTS OF MEDULLARY LESIONS ON ARTERIAL BARORECEPTOR REFLEXES AND RESPONSES TO DISTENSION OF PULMONARY VEIN–LEFT ATRIAL JUNCTIONS IN ANAESTHETIZED DOG

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(*Received 22 November 1976*)

SUMMARY

1. In anaesthetized dogs lesions of 4 mm diameter centred on the obex were placed in the medulla dorsal to the hypoglossal nucleus.

2. Placing of the lesions almost totally abolished the reflex responses to distension of the pulmonary vein–left atrial junctions and to low intensity stimulation of the aortic nerve. The reflex response to high intensity stimulation of the aortic nerve was reduced and the reflex response to carotid artery occlusion remained unaltered.

3. Mid-collicular decerebration did not affect the results.

4. The results are consistent with a hypothesis that afferents from the carotid sinus baroreceptors synapse in the nucleus of the tractus solitarius rostral to the obex, whereas afferent fibres from the aortic baroreceptors and atrial receptors synapse in the intermediate portion of the nucleus of the tractus solitarius close to the obex.

INTRODUCTION

Distension of the pulmonary vein–left atrial junctions by means of small balloons has been shown to cause a reflex increase in heart rate (Ledsome & Linden, 1964) accompanied by small and transient changes in mean arterial pressure (Carswell, Hainsworth & Ledsome, 1970). It has also been shown that pulmonary vein distension is an effective stimulus to left atrial receptors (Linden, 1972). The afferent path for the reflex response is in the vagus nerves and the efferent path is mainly in the cardiac sympathetic nerves (Burkhart & Ledsome, 1974). It is apparent that the cardiovascular responses to distension of the pulmonary vein–left atrial junctions are unlike those of stimulation of the arterial baroreceptors and

it has not been possible to demonstrate any interaction between the response to distension of the carotid sinus and to distension of the pulmonary vein–atrial junctions (Carswell *et al.* 1970). The response to distension of the pulmonary vein–atrial junctions is not quantitatively altered by mid-collicular section (Albrook, Bennion & Ledsome, 1972) or by section of the brain stem in the rostral medulla (Burkhart & Ledsome, 1977). A reflex increase in heart rate in response to this stimulus was also present in dogs with high spinal section (Burkhart & Ledsome, 1974). Thus the major central pathway for the reflex response lies within the medulla.

The experiments described were designed to determine whether the medullary pathways of the reflex response to distension of the pulmonary vein–left atrial junctions could be separated from those of the arterial baroreceptor reflexes. To this end discrete lesions were made close to the dorsal surface of the medulla in the vicinity of the nucleus of the tractus solitarius at the level of the obex. The reflex responses to distension of the pulmonary vein–atrial junctions, carotid occlusion and aortic nerve stimulation were tested.

METHODS

The experiments were carried out on mongrel dogs of either sex, weighing 10–14 kg. The general methods of preparation and recording have been described previously (Burkhart & Ledsome, 1977). Briefly, small balloons were placed in each of three left pulmonary veins to allow distension of the pulmonary vein–left atrial junctions. The dorsal surface of the medulla was exposed by enlarging the foramen magnum and retracting the cerebellum. In some experiments decerebration was performed at the mid-collicular level as previously described (Albrook *et al.* 1972).

Lesions were made in the dorsal medulla using two techniques. In six dogs a lesion was created, centred at the obex, by placing a needle electrode at the obex just below the medullary surface and passing a coagulating current from a surgical coagulator (Birtcher, Blendtome). Because of the muscular movements induced by this type of current these animals were first paralysed by injection of succinyl choline (Glaxo-Allenburys, Scoline, 0·5 mg/kg). In nine dogs a lesion was placed at the obex using a concentric array of electrodes consisting of one central electrode surrounded by a ring of six electrodes each 2 mm from the central electrode. The electrodes were made of 23 gauge stainless-steel and were coated with shrinkable Teflon tubing except for 1 mm at the tips. The tips of the electrodes were placed immediately below the surface of the medulla with the central electrode positioned at the obex. To do this it was necessary to retract the vermis of the cerebellum. A current of 50 μA was passed for 15 sec from a Wyss coagulator (J. Monti, Geneva) between the central electrode and each of the peripheral electrodes in turn. These dogs were not paralysed.

At the end of the experiment the brain stem was removed and fixed by immersion in 10 % formalin for 1 week. The specimen was then frozen and 60 μm sections cut. Alternate sections were mounted and stained using cresyl violet. The site and extent of the lesions was determined by microscopic examination of the sections.

In all experiments the carotid arteries were occluded for periods of 20–30 sec by placing clamps upon the common carotid arteries in the neck. In six dogs the effects of stimulation of the central end of the aortic nerve were examined. The left aortic nerve was identified using the technique described by Edis & Shepherd (1971). After identification the nerve was tied peripherally and stimulated using a pair of silver electrodes enclosed within a light lucite cylinder to prevent spread of current. Square-wave stimulation (Grass Inst. Co. S.8) was applied using low intensity, high frequency (2–4 V, 150 Hz, 0·2 msec), and high intensity, low frequency (6–10 V, 15 Hz, 2 msec) parameters, and was maintained for 10–20 sec.

Experimental protocol

Testing of the response to pulmonary vein distension was always done in the same manner. Following a control period the pulmonary vein balloons were inflated by the injection of 1 ml. saline and the inflation was maintained for 3 min. After deflation the recording was continued for a further 3 min. To allow statistical comparison control values were the average of the heart rate and blood pressure measured over 1 min immediately before balloon inflation and between 2 and 3 min after deflation. The experimental values were measured over the 1 min period 2–3 min after balloon inflation. Immediate changes on balloon inflation and deflation were measured over 10 sec periods, 5–15 sec and 15–25 sec after inflation or deflation. Three such tests were made in each animal in each state, and the average response in each animal was used in statistical comparisons. After completion of these tests, three tests of carotid occlusion were carried out. The heart rates and blood pressures reported are the averages during the 10 sec period in which maximum heart rate occurred as indicated by the cardiotachometer. In six dogs the effects of stimulation of the aortic nerve were examined. Three tests of stimulation with both high and low intensity stimulation parameters were carried out in each animal. Heart rates in the control periods were counted over 30 sec; during stimulation the 10 sec period of lowest or highest heart rate was counted. Mean arterial pressure was averaged over 30 sec in the control periods; during stimulation the lowest or highest mean pressure recorded was measured.

Following these observations, lesions were made in the vicinity of the obex and all observations were repeated. Statistical comparison of the changes in heart rate and blood pressure observed before and after placing the lesions were made using a Student's *t* test for paired data.

RESULTS

The effects of a lesion at the obex on the response to distension of the pulmonary vein–left atrial junctions and carotid occlusion

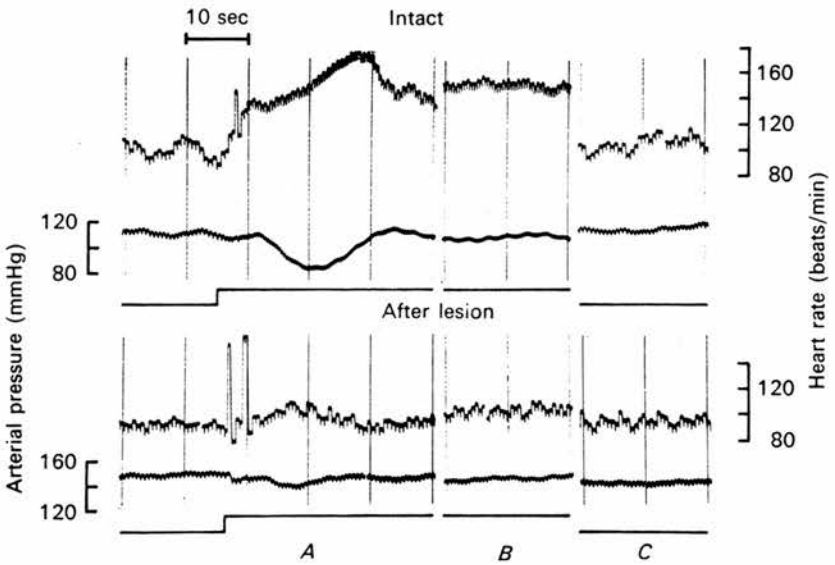
In nine dogs a single lesion (4 mm diameter) was made in the dorsal medulla centred on the obex. In six dogs this was done using a single electrode and a surgical coagulator and in three dogs a concentric electrode and radio-frequency coagulation was used. In all dogs histological examination showed a lesion of approximately 4 mm diameter centred on or slightly caudal to the obex. The lesions extended approximately 2 mm below the dorsal surface of the medulla, just reaching the central canal. The tractus solitarius and the dorsal nucleus of the vagus were destroyed over a length of at least 1 mm rostral to, and 2 mm caudal to the level of

the obex. The hypoglossal nucleus was damaged in some but not all dogs. A region was judged to be intact if the low-power microscopic appearance of the cells in that area was normal. No comment can be made regarding the functional integrity of the cells following the lesion. Pl. 1 is a photomicrograph showing a section through the brain stem from an experiment

TABLE 1. Reflex changes in heart rate and mean arterial pressure in response to distension of the pulmonary vein–left atrial junctions and to occlusion of both common carotid arteries. Results (mean ± S.E. of mean) from nine dogs with 4 mm diameter lesion centred on the obex. The brain stem was otherwise intact.

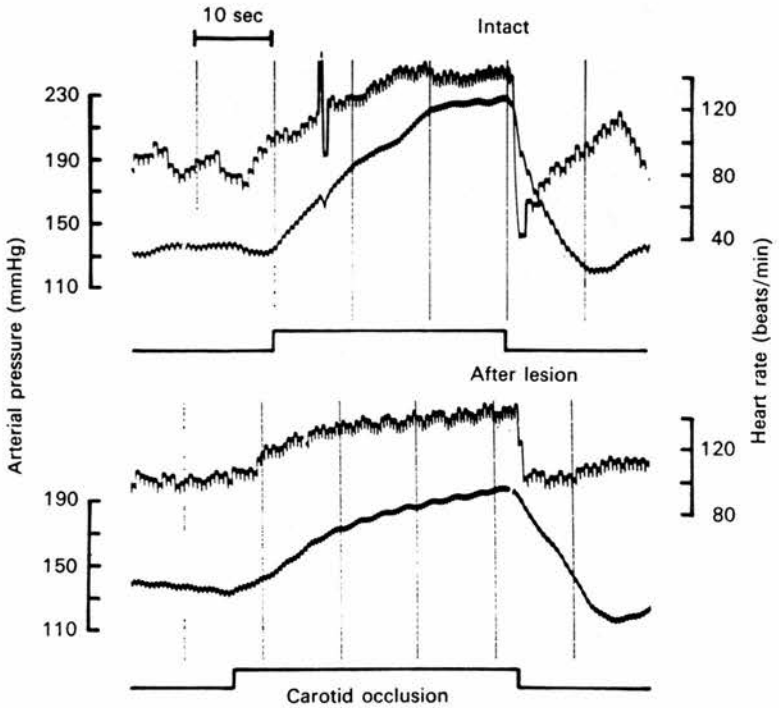
	Heart rate (beats/min)		Arterial pressure (mmHg)	
	Control	Change	Control	Change
Pulmonary vein distension				
Intact	131 ± 7	+29·3 ± 4·8	119·8 ± 8	−1·7 ± 0·9
After lesion	144·7 ± 6·6	+6·2 ± 9·5*	138·1 ± 7	−2·2 ± 1·7
Carotid occlusion				
Intact	125·8 ± 8·4	+12·7 ± 7·2	121·1 ± 8·1	+27·5 ± 5·1
After lesion	149·5 ± 6·2	+15·4 ± 9	131·1 ± 7·3	+31·1 ± 3·4

* Significantly different from the change with brain stem intact ($P < 0{\cdot}05$).



Text-fig. 1. Portions of the record of one experiment. In the first panel (*A*) the time of distension of the pulmonary vein–left atrial junctions is shown by the signal marker. The second panel (*B*) was recorded 2 min later whilst the distension was maintained. The third panel (*C*) was recorded 2 min after removal of the distension. The upper record was obtained before the lesion. The lower record was after the lesion.

in which the lesion was made using a single electrode and coagulating current. Pl. 2 shows a section from an experiment in which the lesion was made using concentric electrodes. As the lesions produced and the results obtained, using the two techniques, were similar, the results have been combined. Immediately before placing of the lesion, with the brain stem exposed, distension of the pulmonary vein–left atrial junctions always



Text-fig. 2. Portions of record from the same dog as in Text-fig. 1. The signal marker indicates a period of carotid occlusion. Upper record obtained before the lesion, lower record after the lesion.

caused an increase in heart rate with no significant change in mean arterial pressure in the steady state (Table 1). In about half of the dogs there was a small transient fall in mean arterial pressure 10–20 sec after balloon distension; an example is shown in Text-fig. 1. Bilateral carotid occlusion always caused a characteristic increase in heart rate and mean arterial pressure (Text-fig. 2, Table 1).

Placing a lesion at the obex invariably caused a marked increase in heart rate and mean arterial pressure. The arterial pressure and heart rate then decreased over the following 10–15 min when a reasonably steady state was reached. No tests were performed until at least 15 min after

placing the lesion. At this time there remained an increase over the control values of heart rate of 10–15 beats/min and of mean arterial pressure of 15–20 mmHg (Table 1). In all nine dogs both the increase in heart rate and any transient decrease in mean arterial pressure which occurred in response to distension of the pulmonary vein–left atrial junctions were significantly reduced (Text-fig. 1, Table 1). In contrast a brisk response to carotid occlusion remained (Text-fig. 2, Table 1).

That the remaining response to carotid occlusion depended upon infracollicular pathways was demonstrated by performing mid-collicular decerebration in four animals. Despite the fact that decerebration caused a decrease in heart rate from 152 to 147 beats/min and a decrease in mean arterial pressure from 145 to 107 mmHg, carotid occlusion was still accompanied by increases in heart rate (22 beats/min before decerebration, 7 beats/min after), and mean arterial pressure (33 mmHg before decerebration, 22 mmHg after). That both efferent sympathetic and vagal pathways to the heart were tonically active and that the activity could be changed by carotid occlusion was also demonstrated. Administration of propranolol (0·5 mg/kg) in five dogs decreased heart rate (from 152 to 125 beats/min) and markedly reduced the increase in heart rate associated with carotid occlusion (from an increase of 8 to only 1 beat/min). After both vagus nerves had been divided in the neck in these five dogs there was an increase in heart rate (from 125 to 141 beats/min) but no longer any increase in heart rate in response to carotid occlusion. The immediate decrease in heart rate on release of carotid occlusion, after placing of the lesion (Text-fig. 2), provides evidence of a vagal efferent component to the response to carotid occlusion following the lesion.

The effects of a lesion at the obex on the response to distension of the pulmonary vein–left atrial junctions, carotid occlusion and stimulation of the aortic nerve

Six dogs were rendered decerebrate at the start of the experiment before any control tests were made. In these dogs a single lesion was made, centred at the obex, using a concentric electrode and a radio-frequency coagulator. The extent of the lesions was similar to that described for the previous group of experiments. In these six animals placing of the lesion at the obex caused an increase in heart rate and mean arterial pressure similar to that observed in the previous nine animals (Tables 1 and 2). In all dogs there was again a marked reduction in the response to distension of the pulmonary vein–left atrial junctions (Table 2). The responses to carotid occlusion remained brisk and although the average changes are somewhat less than before the lesion, the differences are not statistically

significant. Stimulation of the aortic nerve with low intensity, high frequency stimulation caused a decrease in heart rate and a decrease in mean arterial pressure in all six dogs (Table 2). An example of this response is shown in Text-fig. 3. This response was completely absent after a lesion had been placed at the obex. Stimulation of the aortic nerve with higher intensity, low frequency caused an increase in heart rate and blood

TABLE 2. Changes in heart rate and mean arterial pressure induced by distension of the pulmonary vein-left atrial junctions, bilateral carotid occlusion and aortic nerve stimulation before and after placing a lesion at the obex. Results (mean and S.E. of mean) are for six dogs except where noted. All dogs were rendered decerebrate before any measurements.

	Heart rate (beats/min)		Arterial pressure (mmHg)	
	Control	Change	Control	Change
Pulmonary vein distension				
Intact	129·3 ± 15	+19·3 ± 5·8	115·7 ± 7	−3·5 ± 1·5
After lesion	149·5 ± 15	+3 ± 1·1*	134·5 ± 10	−1·9 ± 0·7
Carotid occlusion				
Intact	125·5 ± 15	+30 ± 12	122·2 ± 9	+44 ± 15
After lesion	151·7 ± 16	+16·4 ± 5·4	139 ± 11	+35 ± 7
Aortic nerve stimulation 2–4 V, 150 Hz, 0·2 msec				
Intact	108·5 ± 15	−30 ± 12	119 ± 8	−29·5 ± 7
After lesion	143·5 ± 17	0 ± 2·5*	142·2 ± 10	−3 ± 2*
6–10 V, 15 Hz, 2 msec (three dogs)				
Intact	98 ± 19	+16 ± 3·7	121·3 ± 13	+34·3 ± 5
After lesion	150 ± 11	+8·8 ± 5	139 ± 2·2	+9·2 ± 1·0

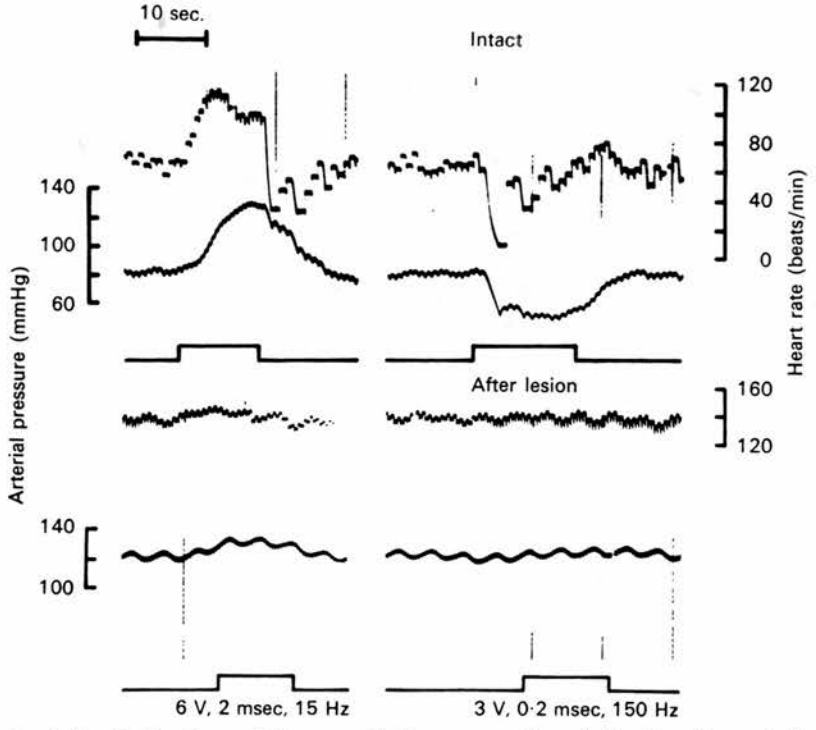
* Significantly different from the change before the lesion ($P < 0·05$).

pressure in three dogs (Table 2, Text-fig. 3); in the other three dogs there was no change or a decrease in heart rate and blood pressure at all stimulus parameters. In the three dogs in which heart rate and blood pressure increased during stimulation this response was still present although reduced in each case after the lesion (Table 2, Text-fig. 3). Statistical analysis was not performed on this small number of dogs.

DISCUSSION

The afferent fibres from cardiovascular receptors which run in the glossopharyngeal and vagus nerves, after entering the medulla, terminate in or close to the nucleus of the tractus solitarius at the level of, or rostral to, the obex (reviewed by Kirchheim, 1976). It has been suggested that

there is a considerable degree of overlap amongst the terminations of the carotid sinus, aortic and superior laryngeal nerves and a high degree of convergence of afferents upon the same unit (Biscoe & Sampson, 1970*a*, *b*; Gabriel & Seller, 1970). However, despite electrophysiological evidence



Text-fig. 3. Portions of the record of one experiment. During the periods indicated by the signal the central end of the left aortic nerve was stimulated at either high intensity, low frequency (left panels) or low intensity, high frequency (right panels). Upper records obtained before lesion, lower records after lesion.

indicating close interaction between the inputs from the glossopharyngeal and vagus nerves, anatomical evidence from degeneration studies (Cottle, 1964) whilst showing some overlap of areas of input in the nucleus of the tractus solitarius indicates the afferents from the carotid sinus in preponderance rostral to the obex, whereas the afferent fibres from the aortic nerve are preponderant in the intermediate portion of the nucleus of the tractus solitarius at the level of the obex. This pattern of distribution is in accord with the careful work of Humphrey (1967) who concluded that fibres from the carotid sinus synapse soon after entering the tractus solitarius in its rostral portion. Humphrey (1967) was unable to record

primary afferent activity from the carotid sinus caudal to a point 0·5 mm rostral to the obex in the cat. As a result of making small lesions in the nucleus of the tractus solitarius 1–2 mm rostral to the obex, he concluded 'few if any baroreceptor afferents involved in the production of sinus cardiovascular reflexes terminate caudal to the level of the lesions'. The small amplitude of evoked multi-unit potentials in the nucleus of the tractus solitarius caudal to the obex led him to suggest that a major fraction of the incoming activity was relayed to structures other than the nucleus of the tractus solitarius. Other investigators have amply confirmed that in the cat lesions in the NTS 1–3 mm rostral to the obex completely prevent all responses to stimulation of the carotid baro- and chemo-receptors (Miura & Reis, 1972).

The pathways within the medulla which are followed by fibres from atrial receptors have been less extensively studied. Vagal afferent fibres which discharge with a cardiovascular rhythm enter the medulla in the more rostral rootlets of the vagus nerve (Bonvallett & Sigg, 1958; von Baumgarten, Koepchen & Aranda, 1959) and section of these rootlets produces degeneration in the intermediate region of the NTS extending slightly caudal to the obex (Cottle, 1964). Modification of neuronal activity in the dog medulla by distension of the pulmonary vein–left atrial junctions has been described as occurring at two sites by Keith, Kidd, Linden & Snow (1975). Neurones in the region of the nucleus of the tractus solitarius had a definite cardiac rhythm and were closely associated with neurones activated by arterial baroreceptors. Other neurones found in the ventral reticular formation deep to the hypoglossal region, did not have a cardiac rhythm and discharged at lower frequencies.

The present observations that a bilateral lesion in the nucleus of the tractus solitarius, extending rostrally and caudally at least 1 mm from the obex abolished the reflex response to distension of the pulmonary vein–atrial junctions whilst leaving intact the response to carotid occlusion are consistent with the hypothesis that fibres from atrial receptors relay in or pass through the intermediate portion of the nucleus of the tractus solitarius close to the obex and that fibres from the carotid sinus baroreceptors relay more rostrally in the nucleus of the tractus solitarius. The observation that the response to low intensity stimulation of the aortic nerve, usually assumed to be due to stimulation of baroreceptor afferents (Edis & Shepherd, 1971), was also abolished, indicates that these vagal afferents also synapse more caudally than the glossopharyngeal afferents as indicated by anatomic studies. The site of termination of aortic chemo receptor afferents in the medulla is unknown; these have been presumed to be responsible for the response to high intensity, low frequency stimulation of the aortic nerve (Edis & Shepherd, 1971). The results

indicate that either a proportion of the fibres mediating the response to high intensity, low frequency stimulation, synapse in the nucleus of the tractus solitarius rostral to the site of the lesion or that some fibres pass ventral or lateral to the site of the lesion before synapsing. The results are unlikely to be due to damage to the cerebellum during the experiment as it has been shown that section of the brain stem in the rostral medulla does not affect the response to pulmonary vein distension (Burkhart & Ledsome, 1977). The observations do not necessarily conflict with electrophysiological studies indicating considerable convergence and interaction between the inputs from the glossopharyngeal and vagus nerves. They provide evidence only that after placing a lesion at the obex a portion remains, of the pathways of the carotid sinus reflex, sufficient to allow the appearance of a significant reflex response. The observations are in agreement with the conclusion of Humphrey (1967) that a major portion of the activity of the carotid sinus baroreceptors is relayed from the site of termination of the primary afferent fibres to structures other than the more caudal regions of the nucleus of the tractus solitarius.

It seems likely that the lesions described, close to the dorsal surface of the medulla, produced their effects by eliminating the terminations and immediate synaptic connexions of the primary afferent fibres from the atrial receptors and the aortic baroreceptors. The second area of projection of atrial receptors described by Keith *et al.* (1975) lying deep to the hypoglossal region is unlikely to have been damaged by the lesion. This area appears to correspond to a similar projection described for the carotid sinus baroreceptors (Humphrey, 1967) and probably represents a polysynaptic projection from the nucleus of the tractus solitarius. However, it may be noted that no projections of sinus afferents could be detected in the medial reticular formation by Lipski, McAllen & Spyer (1975).

It is unlikely that the results described could have been due to interruption of the efferent pathways of the reflex responses. There is good evidence that the vagal efferent fibres to the heart arise mainly from the area of the nucleus ambiguous (Thomas & Calaresu, 1974). Also some evidence is presented that at least in the case of the carotid sinus reflex both vagal and sympathetic efferent pathways to the heart were intact as was the sympathetic efferent pathway to the peripheral nerves. This implies that after synapsing in the nucleus of the tractus solitarius the projections of the sinus afferents mediating all of these effects pass ventrally rather than passing caudally in the tractus solitarius.

Since the afferents stimulated by the distension of the pulmonary vein–left atrial junctions appear to pass close to the afferents from the aortic baroreceptors in the medulla, the question arises as to whether

there is interaction between these inputs. Evidence has been presented (Gabriel & Seller, 1970) that there is interaction at the first synapse between aortic and carotid baroreceptor afferents, despite the apparent separation indicated by the results described here. It seems unlikely that there would be interaction between the atrial receptor input and the aortic baroreceptor input at the first synapse because of the strikingly different response patterns observed on activation of the two receptor areas. We were previously unable to prove any interaction between the atrial receptor input and changes in carotid artery pressure (Carswell *et al.* 1970). However, this point has not been sufficiently examined to allow firm conclusions.

It is of interest that mid-collicular decerebration performed either before or after placement of the lesions had no significant effect on any of the reflex responses. The apparent slight reduction in the responses to carotid occlusion after decerebration in four dogs in the first series can be accounted for by the marked decrease in arterial pressure after decerebration. Decerebration prior to placement of the lesions in the second series did not prevent the rise in arterial pressure which occurred after the lesion was made (Table 2). This is in contrast to the findings of Doba & Reis (1973) who showed that in the unanaesthetized rat decerebration prevented the rise in arterial pressure which followed bilateral lesions in the nucleus of the tractus solitarius. Miura & Reis (1972) also failed to demonstrate a rise in arterial pressure after bilateral lesions in the nucleus of the tractus solitarius in cats which were anaesthetized or rendered decerebrate. These findings led Doba & Reis (1973) to suggest that baroreceptor afferents after terminating in the medulla engage in long loop cardiovascular reflexes with higher brain areas. They also suggested that there is a rostrally situated region necessary for the establishment of hypertension following lesions in the nucleus of the tractus solitarius. The results reported here demonstrate that, in the chloralose anaesthetized dog, supracollicular areas are not essential to the development of hypertension after lesions in the nucleus of the tractus solitarius. This finding should not be interpreted as implying there is no supramedullary modulation of sino-aortic reflexes. Evidence for such supramedullary modulation and also for modulation of sino-aortic reflexes by activity in non-specific viscero-somatic afferents has recently been reviewed (Kirchheim, 1976). There are at present no observations which allow conclusions regarding the possibility of modulation of the reflex responses to pulmonary vein distension by supramedullary structures or by viscero-somatic afferents.

This work was supported by the Medical Research Council of Canada and the British Columbia Heart Foundation.

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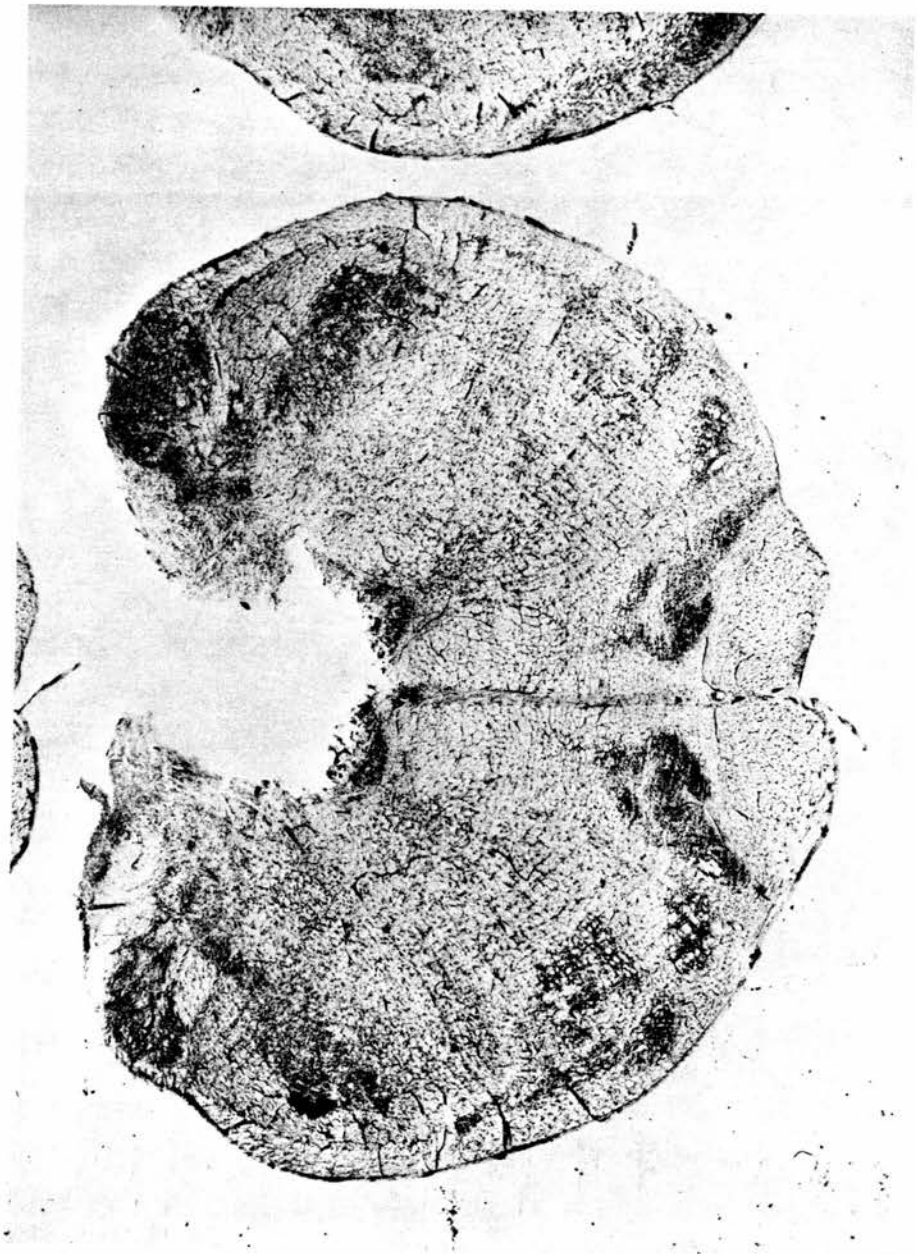

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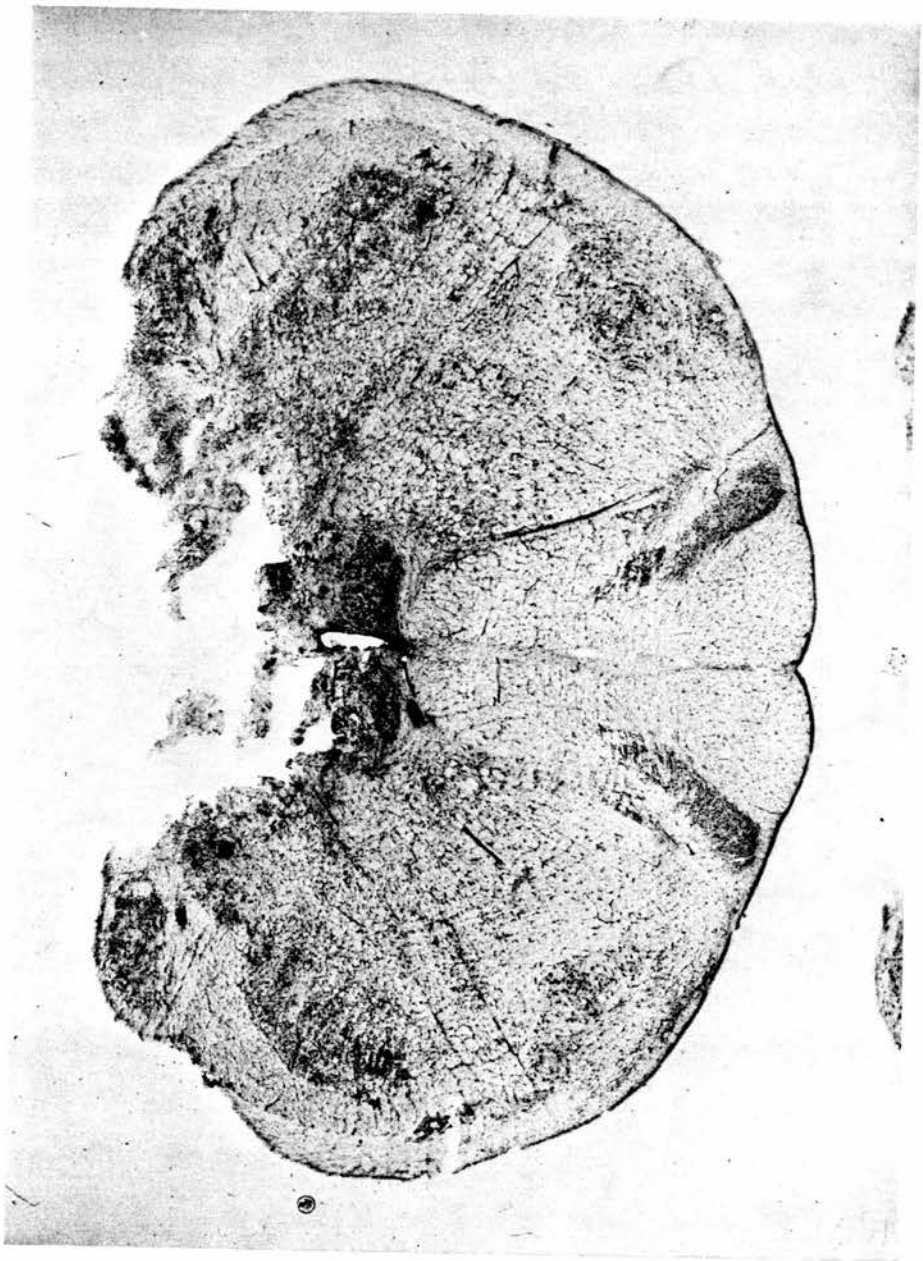

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EXPLANATION OF PLATES

PLATE 1

Photomicrograph of section of the medulla in which a lesion was made close to the obex using a single electrode and a coagulating current. This section is approximately 1 mm caudal to the obex.

PLATE 2

Photomicrograph of a section of the medulla in which a lesion was made close to the obex using a concentric electrode and a radio-frequency current. This section is approximately 1 mm rostral to the obex.

J. Physiol. (1978), **285**, *pp.* 445–453
With 3 text-figures
Printed in Great Britain

THE EFFECT OF DISTENSION OF THE PULMONARY VEIN-ATRIAL JUNCTION ON ACTIVITY OF LEFT ATRIAL RECEPTORS

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(*Received 20 December 1977*)

SUMMARY

1. In anaesthetized dogs, the activity from left atrial receptors with vagal afferent fibres has been examined during distension of small balloons in the pulmonary veins or the application of pulsatile pressures to an isolated perfused pouch of the left atrium.

2. Eleven fibres were found in nineteen dogs. The receptors discharging into these fibres were located, post mortem, to the atrial endocardium and reasons are given for believing that these fibres were myelinated and the receptors were B or Intermediate.

3. Distension of small balloons induced significant increases in the discharge of action potentials from the receptors, the increases were similar to those after infusions of saline or dextran.

4. In the perfused pouch the 'dynamic' thresholds for pulsatile pressure were high as were the mean and pulsatile pressures required to induce bursts of action potentials similar to those observed from atrial receptors in the atrium of normal size. Restoration of the atrium to its normal size resulted in 'dynamic' thresholds and responses to changes in pressure within the normal range. The changes could be explained assuming the Laplace relationship.

5. This evidence supports the conclusion from previous investigations in which these techniques were used, that the increase in discharge in atrial receptors with myelinated vagal fibres could be the cause of the increase in heart rate and urine flow.

INTRODUCTION

From experiments in which parts of the left atrium have been distended it has been argued that excitation of left atrial receptors induces a reflex increase in heart rate and an increase in urine volume (see Linden, 1975, 1976). In these experiments, two methods were used in an attempt to stretch areas of atrial wall containing atrial receptors and thus stimulate them; small balloons inserted into the pulmonary vein-atrial junctions, or a pouch of the left atrium was distended using a pump (Ledsome & Linden, 1967). Although the afferent and efferent nervous pathways were defined in each preparation, in neither case has the relationship

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between the response of the receptors and the stimuli been defined. This investigation examines the response of one group of atrial receptors to the distension of parts of the atrium by the two techniques. A preliminary account has been presented elsewhere (Kidd, Ledsome & Linden, 1966).

METHODS

Dogs (12–25 kg) were anaesthetized and maintained in a state of light anaesthesia and ventilated with a Starling Ideal pump with 40% oxygen humidified at room temperature. Pressures were recorded in the right femoral artery, the main body of the left atrium and the isolated pouch of the left atrium through a middle-left pulmonary vein. The anaesthetic procedures, operative techniques and apparatus have been described previously (Ledsome & Linden, 1964, 1968). The e.c.g. was recorded from leads placed on the forelimb or the chest wall or on the left hind-limb.

The surgical techniques used in inserting the balloons into the pulmonary vein–atrial junction and also in the preparation of the isolated perfused pouch (using a removable clamp) in the atrium have also been described previously (Ledsome & Linden, 1964, 1967). Action potentials were recorded from single functional fibres dissected from the left vagal nerve in the neck (Coleridge, Hemingway, Holmes & Linden, 1957; Coleridge, Coleridge & Kidd, 1964).

During the operative procedures of approximately 2 hr, the animals received an infusion of dextran (Dextraven, Bengers Laboratories Ltd) of approximately 8% of their estimated blood volume (1/13th of the body weight in kg). Rectal temperature was maintained at 37·5 ± 1·0 °C. The arterial pH, pCO_2, pO_2 and bicarbonate concentration were determined by the methods which have been described elsewhere (Norman, Ledsome & Linden, 1965). Throughout, arterial blood pH was maintained by intermittent I.V. infusions of 1·0 M-sodium bicarbonate solution. The arterial pCO_2 was maintained by adjusting the stroke of the respiratory pump. Mean values are quoted in association with standard deviation (S.D.) throughout.

Experimental procedure

Identification and location of receptors

In both series, fibres whose activity was consistently increased by distension of the pulmonary vein–left atrial junctions, were used for further analysis provided they satisfied the following criteria:

(*a*) the application of pulsatile pressure to the pouch or inflation of the balloon should induce consistent increases in the discharge from the receptors;

(*b*) steady distension should induce a sustained increase in discharge which was maintained throughout the period of distension; and finally

(*c*) activity in a fibre should not be markedly influenced by large changes in tidal volume and should not otherwise exhibit characteristics of a pulmonary stretch receptor.

Fibres whose activity did not satisfy all these criteria were not examined in detail.

Records were taken of action potentials evoked in single fibre preparations by distension of the balloons at the pulmonary vein–atrial junctions and by distension of the pouch with a range of steady and pulsatile pressures. In each experiment the response of a receptor in the pouch was examined and the clamp on the left atrium (Ledsome & Linden, 1967) was then removed so that the atrium was restored to its normal size and records were made as atrial pressure was increased by I.V. infusions of dextran or 0·9% sodium chloride solution. In all animals, the location of the receptor was finally defined precisely with a fine glass probe in the widely opened atrium after the animal had been killed (Coleridge *et al.* 1957; Coleridge *et al.* 1964).

RESULTS

Experiments were carried out on a total of nineteen dogs; in six animals, small balloons were used to excite the left atrial receptors and in thirteen animals, a pouch in the left atrium was created and distending pressures applied. The activity of eleven receptors was studied, and the patterns of spontaneous activity and the

responses to infusions of saline were those observed from receptors of the type B or Intermediate variety (Paintal, 1973). The arterial pH was 7·38 (mean; range 7·37–7·41); $p_{a.CO_2}$ was maintained at 40·2 mmHg (mean; range 38–42 mmHg), $p_{a.O_2}$ was 196 mmHg (mean; range 102–245 mmHg).

Distension of balloons at pulmonary vein–atrial junctions

Distension of a balloon in the upper or middle left pulmonary vein–atrial junction with 1 ml. saline induced an immediate increase in the impulse activity recorded from each of the left atrial receptors tested (Fig. 1). During the control period

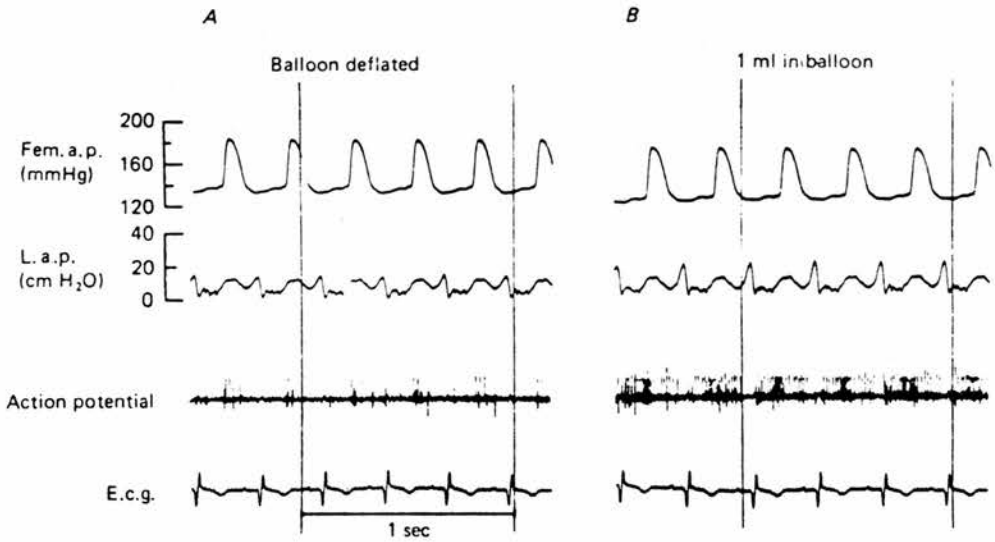

Fig. 1. Effect of balloon distension (1 ml.) on left atrial receptor activity. From above down: femoral arterial blood pressure (mmHg); left atrial pressure (cm H_2O), action potentials from atrial receptors, electrocardiogram. *A*, record with balloons not distended. *B*, 2 min after distension of balloon in upper pulmonary vein with 1 ml. saline.

3–4 impulses occurred in each cardiac cycle and distension resulted in an increase to 20·8 impulses/cycle (mean; $\pm 0{\cdot}94$). The activity still had a cardiac rhythm and interspike intervals were of the same order as those recorded from receptors in the normal intact atrium when exposed to infusions of saline (Fig. 2*B*).

The response to distension was consistent and reproducible. Thus, the receptor illustrated in Fig. 1 responded to five distensions over a 90 min period with mean increases in impulse frequency which ranged from 18·4 to 22·5 impulses/cycle. Over-all, from receptors on the left side of the atrium injection of 1 ml. saline into either the upper or middle pulmonary vein balloon induced a mean increase in frequency to 36·0 impulses/cycle (range $17{\cdot}9 \pm 1{\cdot}5$–$55{\cdot}9 \pm 6{\cdot}7$) from a mean control level of 6·3 impulses/cycle (range $2{\cdot}2 \pm 0{\cdot}4$–$14{\cdot}6 \pm 2{\cdot}9$); a single receptor on the right side of the left atrium increased its activity from $2{\cdot}8 \pm 0{\cdot}5$–$4{\cdot}4 \pm 0{\cdot}6$ impulses/cycle. Thus the increase ranged between 2 and 11 times the control level. The increase in activity from all receptors developed immediately and occurred without significant changes in either heart rate or mean systolic arterial blood

pressure. That changes in heart rate were not observed is probably a reflexion of damage to the vagus during the search for single fibres.

During the earlier studies of reflex responses the stimulus was maintained for 2–3 min (e.g. Ledsome & Linden, 1964) and the responses of five receptors to sustained distension of 2 min were therefore determined. Distension of the balloon increased the impulse traffic from three receptors which was maintained for the period of distension whereas activity from two receptors diminished by 1–1·5 min, although a significant increase remained at the end of 2 min. This reduction in activity may not entirely represent adaptation, as is usually described for receptors since, as the balloon moves in relation to the wall of the left atrium the receptors are exposed to a dynamic stimulus. This effect can be observed as a cardiac modulation in the evoked activity (Fig. 1*B*).

Responses from left pouch preparation

The intention of this section was to define the response of left atrial receptors to the range of pressures applied during the previous study of reflex responses (Ledsome & Linden, 1967). Action potentials were recorded from five vagal fibres; in eight dogs no fibres were encountered which satisfied the criteria outlined (see Methods). For each receptor static and pulsatile pressures were applied and 'dynamic' threshold (defined as the pressure at which the first impulse was evoked) together with the number and frequency of action potentials, was examined. With a pulsatile stimulus similar to that used in the reflex studies, each receptor produced action potentials during the rising phase of the pressure pulse. This is illustrated in Fig. 2*A*, as the pouch pressure increased to approximately 70 cm H_2O, a burst of 8–10 impulses was evoked and the threshold was 27 cm H_2O. Aggregated data for all receptors shows that a pulsatile pressure of 48·0 cm H_2O (mean; range 32·0 ± 17·6–75·4 ± 3·2) on a mean pressure of 36·8 cm H_2O (range 25·5 ± 8·7–52·3 ± 8·4) induced 16·3 impulses/pressure pulse (mean; range 10·6 ± 1·6–29·0 ± 1·4). The 'dynamic' thresholds were high (mean 39·4 cm H_2O; range 20·5 ± 5·2–59·7 ± 6·2 cm H_2O). Variations in action potential frequency were induced by changing mean and pulsatile pressures and repeated application of pulsatile pressures induced consistent responses.

Responses in 'pouch' and 'open' left atrium

The responses of each receptor were examined with the clamp in position and then with the clamp removed so that receptor activity was studied in the whole atrium under more 'normal' physiological conditions (Fig. 2).

The data on the responses of all receptors over the whole range of pressures and evoked response are summarized in Table 1. In the 'open' atrium (clamp off) a mean atrial pressure of 13·3 cm H_2O (range 6·3 ± 0·46–21·0 ± 1·1) and a '*v*' wave amplitude of 7·2 cm H_2O (range 4·1 ± 0·7–9·5 ± 1·70) evoked 6·4 action potentials/150 msec. cardiac cycle (range 2·8 ± 0·6–10·1 ± 0·9) with a mean 'dynamic' threshold ('*v*' wave) of 14·3 cm H_2O (range 6·8 ± 0·70–24·9 ± 3·6 cm H_2O). Comparison of the responses for each receptor shows that invariably pressures were significantly lower in the 'open' atrium preparation and 'dynamic' thresholds were lower by a factor of 2–5. These differences are further emphasized when recordings made in the open and closed atria during which a similar number of evoked action

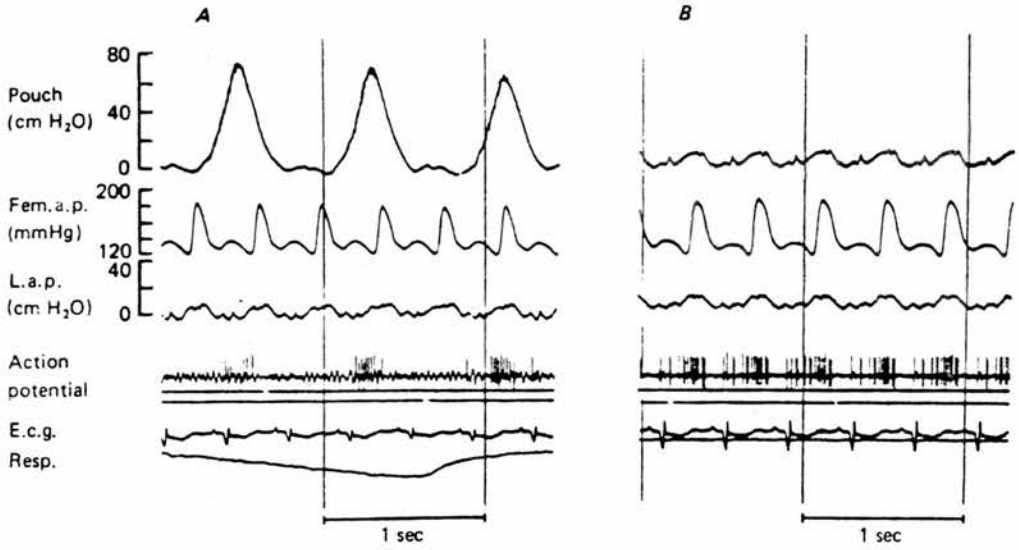

Fig. 2. Action potentials and pressure from an atrial receptor when in wall of pouch and in the 'open' atrium. From above down: pressure in pouch (cm H_2O); femoral arterial pressure (mmHg), left atrial pressure (cm H_2O), action potentials from receptor, electrocardiogram, tracheal pressure (inflation upwards). *A*, clamp across left atrium and pressure pulse applied to pouch. *B*, activity from same receptor when clamp removed to restore pouch to original size ('open' atrium) and after infusion of 200 ml. 0·9% sodium chloride solution. When this section of record was taken, inflation was briefly interrupted.

TABLE 1. Comparison of responses to pressure (p) changes in 'pouch' and 'open' atrium (mean and S.D.)

Expt.	Mean p (mmHg)	Pulse p (mmHg)	Threshold (mmHg)	Impulses/ 150 msec	Impulses/ pulse
		A Clamp on (pouch)			
30/64	25·7 ± 3·9	75·4 ± 3·2	36·3 ± 1·2	10·6 ± 1·6	10·6 ± 1·6
39/64 (1)	52·5 ± 8·4	45·9 ± 8·7	47·7 ± 3·8	8·5 ± 0·8	14·7 ± 2·3
(2)	52·5 ± 8·4	45·9 ± 8·7	59·7 ± 6·2	11·0 ± 2·7	12·7 ± 2·5
34/65	27·8 ± 7·8	41·1 ± 11·9	20·5 ± 5·2	12·3 ± 2·8	29·0 ± 1·4
36/65	25·5 ± 8·7	32·0 ± 17·6	32·3 ± 4·2	7·6 ± 0·8	14·6 ± 11·9
Mean	36·8	48·0	39·4	10·0	16·3
		B Clamp off (open)			
30/64	6·7 ± 0·8	8·3 ± 0·80	6·8 ± 0·70	9·9 ± 0·5	
39/64 (1)	19·6 ± 1·3	9·5 ± 1·70	18·1 ± 1·2	6·0 ± 1·2	
(2)	21·0 ± 1·1	9·4 ± 1·5	24·9 ± 3·6	3·1 ± 1·6	
34/65	6·3 ± 0·95	4·8 ± 0·95	7·0 ± 1·0	10·1 ± 0·9	
36/65	12·6 ± 0·4	4·1 ± 0·7	14·5 ± 0·4	2·8 ± 0·6	
Mean	13·3	7·2	14·3	6·4	

potentials are compared. Action potentials evoked during pressure pulses in the pouch were compared with those during the '*v*' wave of the left atrial pressure pulses during infusion of dextran or saline (Fig. 2*A*, *B*). To allow comparisons between 'open' and 'closed' atrium which were independent of varying duration of the pressure pulses an index of action potential activity (impulses/150 msec) was chosen. For every receptor encountered the mean pressures, pulse pressures required to induce a similar number of action potentials (impulses/150 msec) in either the 'pouch' (clamp on) or 'open' (clamp off) left atrium, together with threshold pressures, are shown in Fig. 3. In each preparation, the pressures were significantly lower in the 'open' atrium.

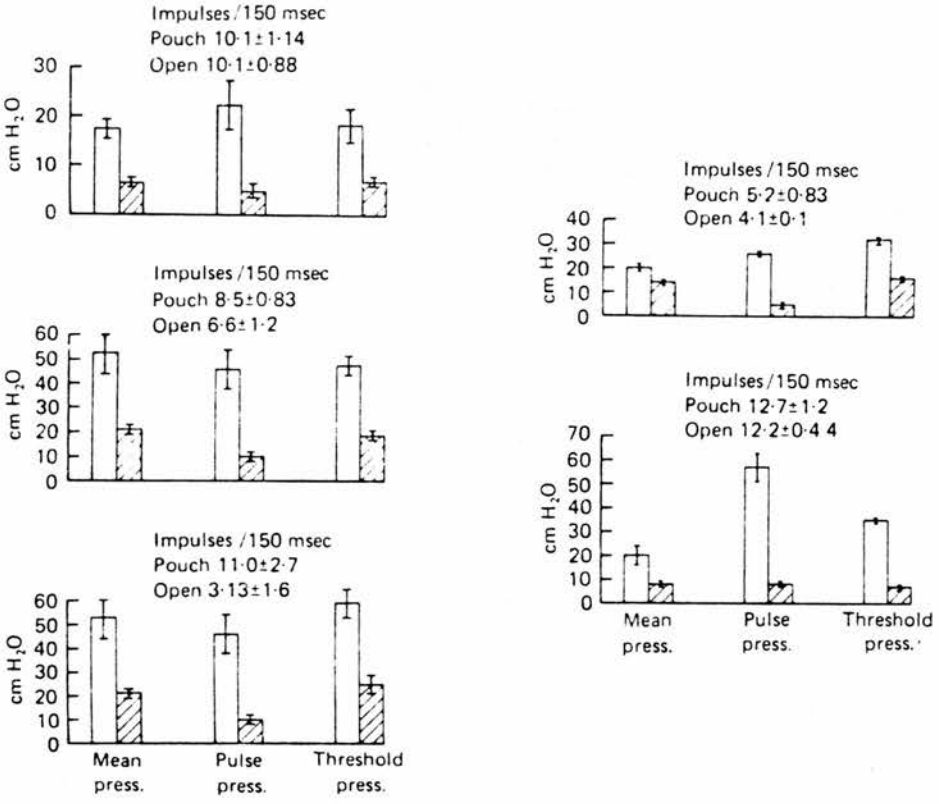

Fig. 3. Comparison for each of five receptors of pressures required to induce a similar number of impulses during the first 150 msec of a pressure pulse. The values refer to impulses per 150 msec when the receptor was in the wall of the pouch or the 'open' atrium. The blocks give value for mean pressure, pulse pressure and dynamic thresholds in the pouch (not shaded) and 'open' atrium (shaded). For each receptor the pressures are lower in the 'open' atrium than in the pouch.

In conclusion, therefore the creation of a pouch of the left atrium causes the threshold of the receptor to be increased and its sensitivity to applied pressures to be significantly diminished.

Location of receptors

In the balloon series, six receptors were finally located in six dogs; in the experiments in which the pouch was distended five atrial receptors were located in thirteen dogs. Over-all, eight receptors were in the anterior and posterior walls of the atrium close to the middle and upper pulmonary vein–atrial junctions or the region between the base of the atrial appendage and the upper pulmonary vein. A single receptor (in the balloon series) was in the wall of the right middle pulmonary vein. The remaining two receptors were on the anterior wall of the left atrium close to the middle and upper pulmonary vein. In each case the receptive area was a circumscribed area (1–2 mm diam.) most easily activated from the endocardial surface.

DISCUSSION

The major objective of this study was to examine the increases in vagal nerve fibre activity which occur in response to two previously used stimuli, distension of small balloons in the pulmonary vein–atrial junctions and of a pouch of the left atrium, each of which had been shown to cause an increase in heart rate and urine flow. As described in Methods the first fibres encountered during dissection which satisfied stated criteria were examined. In the event all receptors from the atrium proved to be of Paintal type B or Intermediate atrial varieties.

Although conduction velocities were not determined, several lines of evidence suggest strongly that the receptors studied were attached to vagal myelinated fibres. Receptors were located discretely in regions which have previously been delineated, on both histological and electrophysiological grounds, as those in which receptors with myelinated vagal afferent fibres are to be expected (Coleridge *et al.* 1957; Coleridge *et al.* 1964; Nonidez, 1937). The patterns of discharge before distension or after removal of the clamp were characteristic of those for myelinated fibres attached to type B and Intermediate receptors (Paintal, 1973). The number of impulses and the pattern of discharge during distension of the balloons or application of pressure to the pouch were characteristic of those observed from atrial receptors with vagal myelinated fibres during infusion and the expansion of the blood volume. The responses to distension of the balloons were consistent and the increase persisted in all fibres for at least 2 min, although in two of the six fibres the maintained activity was less than the initial discharge. By contrast, atrial receptors with non-myelinated vagal afferent fibres have different characteristics. Their spontaneous activity is irregular, of low frequency and is usually without cardiac modulation. In addition, they have higher thresholds and a lower discharge frequency/unit pressure relationship than receptors with myelinated afferent fibres (Coleridge, Coleridge, Dangel, Kidd, Luck & Sleight, 1973; Thoren, 1976). No claim is made that only this type of receptor is stimulated by these techniques, only that these receptors are stimulated in a manner which suggests that they could be involved in the previously reported reflex increases in heart rate and urine flow. Further support for this conclusion arises from the observation that no pure type A receptors are to be found in the atrial endocardium (Kappagoda, Linden & Mary, 1977).

The results of the balloon experiments allow several important conclusions:

inflation of small balloons, an apparently unphysiological stimulus, induces a discharge of action potentials from these left atrial receptors which has many of the characteristics of the activity evoked by the more physiological methods of infusing blood or dextran. Thus the central neurones in the reflex pathways are receiving a physiological pattern of activity from the periphery.

This response is reproducible and involves a significant number of receptors including a modest increase in activity from some receptors on the right side of the left atrium. An over-all increase of at least 2–11-fold in the afferent barrage impinging on the central nervous system was evoked; such an increase can be expected under physiologically evoked circumstances.

Further evidence that these receptors could be involved in the heart rate response derives from a consideration of the mean and pulsatile pressures within the pouch with the resultant impulse frequencies and differences in threshold observed between the pouch and reconstituted whole left atrium. High pressures (15–70 cm H_2O) were required in the previous study (Ledsome & Linden, 1967) to evoke a moderate reflex increase in heart rate of 10·3 beats/min (mean; range 3–27). In this investigation, similar high pressures of the order of 25–50 cm H_2O were needed to induce a moderate increase in discharge of impulses from atrial receptors, up to 8–12 impulses/pressure pulse, at similar rates of pulsation. It should be noted that the rates of rise of the '*v*' wave of the left atrial pressure pulse were significantly lower in the 'open' atrium as compared with the applied pressure pulses when the clamp was in position.

These differences in the responses of receptors in the pouch compared with the 'open' atrium, are compatible with the explanation previously advanced to account for the high pouch pressures required to elicit a small increase in heart rate (Ledsome & Linden, 1967). It was suggested that when the pouch was created the radius of the distended part of the atrium diminished and, in accordance with the Laplace relationship, a proportionately greater pressure would be required to guarantee the same circumferential tension in the atrial wall; the interpolation of appropriate values for the radius of the atrial wall with and without the clamp predicted that the changes in pressure required to induce similar wall tensions were of the same order of magnitude as those which were applied in this study to induce equivalent changes in impulse activity.

It is concluded that both methods of distension of the atrium result in the excitation of atrial receptors attached to myelinated vagal afferent fibres and the evidence supports the conclusion from previous investigations in which these techniques were used and reflex changes in heart rate and urine flow observed (Ledsome & Linden, 1964, 1968) that the increases in discharge of atrial receptors could be the cause of the increase in heart rate.

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Time course of changes in plasma vasopressin during atrial distension[1]

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Received November 20, 1981

LEDSOME, J. R., N. WILSON, and J. NGSEE. 1982. Time course of changes in plasma vasopressin during atrial distension. Can. J. Physiol. Pharmacol. **60**: 1210–1218.

Distension of the left atrium in chloralose anaesthetized dogs causes a diuresis and dilution of the urine. It has been reported previously that if distension of the atrium is maintained then urine flow reaches a peak after 50 min and then declines. A radioimmunoassay was used to measure plasma arginine vasopressin (AVP) at 10-min intervals before, during, and after atrial distension for 90 min. Plasma AVP decreased during atrial distension and did not increase until after the atrial distension was removed. Urine volume and free-water clearance increased and urine osmolality decreased, to reach maximum changes after 50 min. Although there was then a decline in some experiments, after reaching the peak changes, the mean values of the group did not show any statistically significant decline. Thus the urinary changes were also present for the 90 min of left atrial distension. The results are consistent with the hypothesis that the diuretic response to left atrial distension is dependent upon decreased release of AVP from the neurohypophysis.

LEDSOME, J. R., N. WILSON et J. NGSEE. 1982. Time course of changes in plasma vasopressin during atrial distension. Can. J. Physiol. Pharmacol. **60**: 1210–1218.

La distension de l'oreillette gauche de chiens anesthésiés au chloralose provoque une diurèse et une dilution de l'urine. Il a déjà été dit que lorsque l'on maintenait la distension de l'oreillette, le débit urinaire atteignait une crête après 50 min pour ensuite diminuer. On a utilisé un dosage radioimmunologique pour mesurer la vasopressine d'arginine plasmatique (AVP) à intervalles de 10 min, avant, pendant et après une distension auriculaire de 90 min. L'AVP plasmatique diminua pendant la distension auriculaire et n'augmenta qu'après l'arrêt de la distension auriculaire. Le volume urinaire et la clearance de l'eau libre augmentèrent et l'osmolalité de l'urine diminua, atteignant des variations maximales après 50 min. Lors de certaines expériences, on observa qu'après avoir atteint les variations de crête, les valeurs accusèrent une baisse; toutefois, les valeurs moyennes du groupe ne montrèrent aucune diminution statistiquement significative. Ainsi, les variations urinaires étaient aussi présentes pendant les 90 min de la distension de l'oreillette gauche. Les résultats concordent avec l'hypothèse qui veut que la réponse diurétique à la distension auriculaire gauche dépende d'une libération plus faible d'AVP de la neurohypophyse.
[Traduit par le journal]

Introduction

Distension of the left atrium in anaesthetized dogs causes an increase in urine flow (Henry *et al.* 1956; Ledsome *et al.* 1961; Arndt *et al.* 1963). In the majority of experiments atrial distension increases urine flow within 5–10 min of the start of the atrial distension. The period of atrial distension has usually been 30 min. Under these circumstances urine flow reaches its peak in the third 10-min period of atrial distension or in the 10-min period immediately following release of the atrial distension. The increase in urine flow is accompanied by a decrease in urine osmolality and an increase in free-water clearance; the changes each have a similar time course.

Distension of the left atrium for periods longer than 30 min results in urine flow reaching a peak after 40–50 min and then declining. This observation was first recorded by Henry *et al.* (1956) and later confirmed by Ledsome *et al.* (1961) and by Shu'ayb *et al.* (1965). The time course of the response to 90 min of atrial distension was described in detail by Lawrence *et al.* (1973), who observed a diminution in the response after 40 min of atrial distension. Although free-water clearance and urine osmolality tended to return to control values after reaching peak changes at 40 min, both of these variables remained significantly different from the control values until after atrial distension was released.

It has been suggested recently that when atrial distension is maintained for a period of 60 min the rate of impulse discharge from atrial receptors, for a given atrial pressure, is diminished (Kappagoda and Padsha 1981). This could provide one explanation for a diminution in the urinary response with time. However, the mechanisms by which left atrial distension causes a diuresis are not proven. Gauer and Henry (1976) have summarized the evidence that the diuretic response may be due to a decrease in the concentration of vasopressin in the circulating blood and their view is widely accepted. This hypothesis has been questioned by others (Goetz *et al.* 1975; Kappagoda *et al.* 1974). Direct measurements of vasopressin concentration in the plasma have usually shown decreases in vasopressin con-

[1]Supported by the Medical Research Council of Canada and the British Columbia Heart Foundation.

0008-4212/82/091210-09$01.00/0

centration during atrial distension (Johnson *et al.* 1969; de Torrente *et al.* 1975; Zucker *et al.* 1979) although some workers have been unable to detect such changes (Kappagoda *et al.* 1974). Changes in blood antidiuretic hormone (ADH) were examined by Shu'ayb *et al.* (1965) during periods of atrial distension of 60 min or more. They stated that "Both ADH and urinary flow started to return to the preinflation level in 30 to 40 min although the atrial pressure was still elevated." This relationship was not evident in the majority of their figures.

The experiments to be described examine the changes in urinary excretion and plasma arginine vasopressin (AVP) concentration during atrial distension for periods of 90 min. If the stimulus to atrial receptors declines with time and if the diuretic response to atrial distension is due to a decrease in release of ADH from the neurohypophysis, it would be expected that changes in plasma ADH would precede or parallel the urinary response.

Methods

Nine dogs of 14–22 kg (average, 18.5) were given a subcutaneous injection of morphine sulphate (0.5 mg·kg^{-1}). One hour later, a catheter was introduced, under local anaesthesia (1% mepivacaine hydrochloride, Winthrop Laboratories, Aurora, Ont.), through a saphenous vein. Anaesthesia was induced with α-chloralose (1 g·100 mL^{-1}, 0.6% w/v NaCl; 10 mL·kg body weight^{-1}; B.D.H. Chemicals). After completion of the surgical procedures, a steady state of anaesthesia was maintained by the continuous infusion of a chloralose solution (0.5 g·100 mL^{-1}, 0.6% w/v NaCl) at a rate of 2 mL·min^{-1}.

As soon as possible after the induction of anaesthesia, artificial respiration was started with 40% O_2 in N_2 supplied from a respiration pump (Harvard Apparatus Co., model 614) the rate (about 14/min) and stroke volume (about 50 mL·4 kg body weight^{-1}) of which were adjusted to approximately equal that of the animal's spontaneous respiration. When the chest was opened a resistance equivalent to 3 cmH_2O was provided by an exhalation valve (Ohio Chemical). At intervals during the procedures samples of arterial blood were taken and pH, P_{CO_2}, and P_{O_2} were measured using appropriate electrodes (Instrumentation Laboratories model 127). Before the experimental period began adjustments were made to the respiratory pump or infusions of sodium bicarbonate solution (1 *M*) were given to bring Pa_{CO_2} to between 35 and 40 mmHg (1 mmHg = 133.322 Pa) and pH within the range 7.3–7.4.

Each ureter was cannulated through a flank incision and urine was collected at 10-min intervals. The left side of the chest was opened in the fifth intercostal space and a balloon placed in the left atrium as described previously (Ledsome and Mason 1972). Femoral arterial pressure and left atrial pressure were recorded through 150 mm lengths of Teflon tubing (1-mm bore). To each cannula was attached a strain gauge (Statham Instrument Co., P_{23}Db) and after direct current amplification the pressure was recorded on an ultraviolet light recorder (Honeywell, Visicorder 1508). Mean pressures were obtained electrically.

During the surgical procedures, about 2 h, the animals received an infusion of dextran 70 in 0.9% NaCl (Pharmacia Ltd., Dorval, P.Q.) 100 mL for each 13 kg of body weight. The electrocardiogram was recorded from leads on the hind limb and chest wall. Heart rates were counted from the electrocardiogram over periods of at least 30 s. Oesophageal temperature was maintained at 37 ± 1.0°C using a heated table and temperature controller (Yellow Springs Instrument Co.).

Analytical methods

Samples of arterial blood (10 mL) were taken into clean dry syringes; the volume of blood removed was immediately replaced with dextran. One part (7 mL) of the blood was transferred into EDTA tubes (Vacutainer) the remainder (3 mL) was mixed with 2 drops of heparin (Heparin sodium, Nutritional Biochemicals, 1000 units/mL) and centrifuged. The EDTA tubes were placed on ice and centrifuged in a cold room (4°C) immediately following completion of the experiment. Heparinized plasma and urine were analyzed for sodium and potassium using a flame photometer (Instrumentation Laboratories Inc.). Urine and plasma osmolality were measured by freezing-point depression (Osmette, Precision Systems). The average difference between duplicate estimations of urine and plasma osmolality was 4.4 mosmol·kg^{-1}, and 2.6 mosmol·kg^{-1}, respectively.

Plasma from the EDTA tubes was stored at −20°C and later used for radioimmunoassay (RIA) for arginine vasopressin (AVP). The period of storage did not exceed 2 weeks. Prior to RIA plasma samples were extracted with organic solvents as described by Robertson *et al.* (1973) to remove nonspecific interference factors. Modifications of the method consisted of the following: (*i*) evaporation of the organic phase was under a N_2 stream, test tubes resting in the ice bath of the evaporator (N-EVAP, model 10, Organomation Associates, Shrewsbury, MA); (*ii*) 2.0-mL plasma specimens were used with corresponding increase in solvent volumes; (*iii*) the evaporation time was increased to 2 h or more; and (*iv*) the volume of the remaining concentrate was determined by weighing. Up to 36 specimens were extracted at one time, including three recovery controls, a reagent blank, and a vasopressin-free plasma (Burget and Wilson 1979). The resulting extracts (ca. 1.7 mL) were stored at −20°C and the RIA conducted within 1 week. RIA was also performed on the same plasma samples without prior extraction. The organic solvent extraction procedure resulted in the removal of the nonspecific interference factors, as well as in losses of the hormone itself. The quantitation of this hormone loss was performed by the simultaneous extraction of a known concentration of vasopressin (recovery controls). The extent of the nonspecific interference could be obtained only from a comparison of vasopressin measurements in the same samples prior to and following the extraction procedure. The presence of nonspecific interference factors resulted in immunoreactive vasopressin values which were falsely elevated in the unextracted samples (Robertson *et al.* 1973). However, the relationship between the extracted and unextracted values appeared fairly constant in any one dog (see below), thus rendering the measurements of the relative changes in vasopressin concentration valid on unextracted samples.

RIA for plasma ADH was a modification of the one reported earlier for measurement of this hormone in tissue extracts (Keeler and Wilson 1976; Wilson and Ngsee 1980). The anti-AVP serum (No. GP-15) used in this study was obtained from a guinea pig by the method previously described for oxytocin (Wilson and Greenhouse 1976). GP-15 showed no cross-reactivity with oxytocin, 4-ser-9 ileu oxytocin, arginine vasotocin, or angiotensin I. The final antiserum dilution in these experiments was 1 : 60 000.

AVP standards were prepared from pituitary extract (USP pituitary extract, 1.9 U/mL), a gift from Dr. R. E. Weitzman. This standard was compared with synthetic AVP (Spectrum Laboratories, Los Angeles, CA, lot 208062), to express AVP values on weight basis. Monoiodinated AVP was prepared from synthetic vasopressin (Sigma, grade VIII). The specific activity of ^{125}I-labelled AVP was 1020 μCi/μg (1 Ci = 37 GBq).

All analyses were conducted in triplicate. Unknowns were analyzed in the presence and in the absence of antiserum (six test tubes per unknown sample) to account for nonspecific binding of ^{125}I-labelled AVP. The volumes of plasma, or plasma extract, varied between 100 and 300 μL per tube, depending on hormone concentration. Incubation was carried out for 5 days: 1 or 2 days preincubation and 3 or 4 days following the addition of ^{125}I-labelled AVP. The total incubation volume was 1.0 mL, and all assay components were diluted in 0.15 *M* phosphate buffer, pH 7.2.

Separation of bound from free hormone was done using dextran-coated charcoal, and both bound and free fractions were counted (automatic gamma counting system, Nuclear Chicago, 1185 C series). The data were printed on tape using an online teletype, and analyzed by a computer using the logit plot, and the quality contol system described by Rodbard *et al.* (1968). The limit of detection (sensitivity), defined as 80% of the maximum binding was 0.59 pg AVP (±0.02 SE, n = 47). The upper limit of detection defined as 20% of maximum binding was 11.08 pg AVP (±0.45 SE, n = 54). Intraassay variability at the 2.5 pg level was 4.5%, interassay variability at the 2.5 pg level was 7.36%. Values were recorded as below assay sensitivity for less than 1.4 pg·mL^{-1} using 200-μL samples or less than 1.0 pg·mL^{-1} using 300-μL samples.

At the time the experiments were performed the RIA was not sufficiently sensitive to detect plasma AVP in all of the extracted samples, particularly those obtained during atrial distension. Results are therefore presented in this paper of AVP measurements in unextracted plasma. The relationship between the extracted (corrected for recovery) and unextracted plasma AVP was examined using sequential experimental samples from five dogs. Eighty-five pairs of measurements of plasma AVP obtained before and after extraction were used for this analysis. In these experiments the average AVP recovery (from recovery controls) was 85% (range 75.2–93.7). The results are presented in Fig. 1. In three dogs the relationship between extracted and unextracted plasma AVP was similar (line 1). This line is described by the equation: unextracted plasma AVP = 6.1 + (1.14 × extracted plasma AVP). The correlation coefficient for this line (r) was 0.95. In another dog (line 2) the relationship had a similar intercept (6.5) but the slope of the line was different (2.47). There was again a high correlation between the extracted and unextracted plasma values (r = 0.97). In the fifth dog (line 3) the intercept was at the origin and the slope was 1.47. Again the correlation was high (r = 0.97); in this dog there was presumably little nonspecific interference. Thus, in an individual dog there is a high correlation between the extracted and unextracted plasma AVP. However, there may be marked differences, between dogs, in both the slope of the relationship and the intercept, which prevents prediction of extracted plasma values from the unextracted plasma measurements without first establishing the relationship in each animal. The high degree of correlation between the extracted and unextracted plasma values does mean that measurements on the unextracted plasma can be used to show changes in plasma AVP.

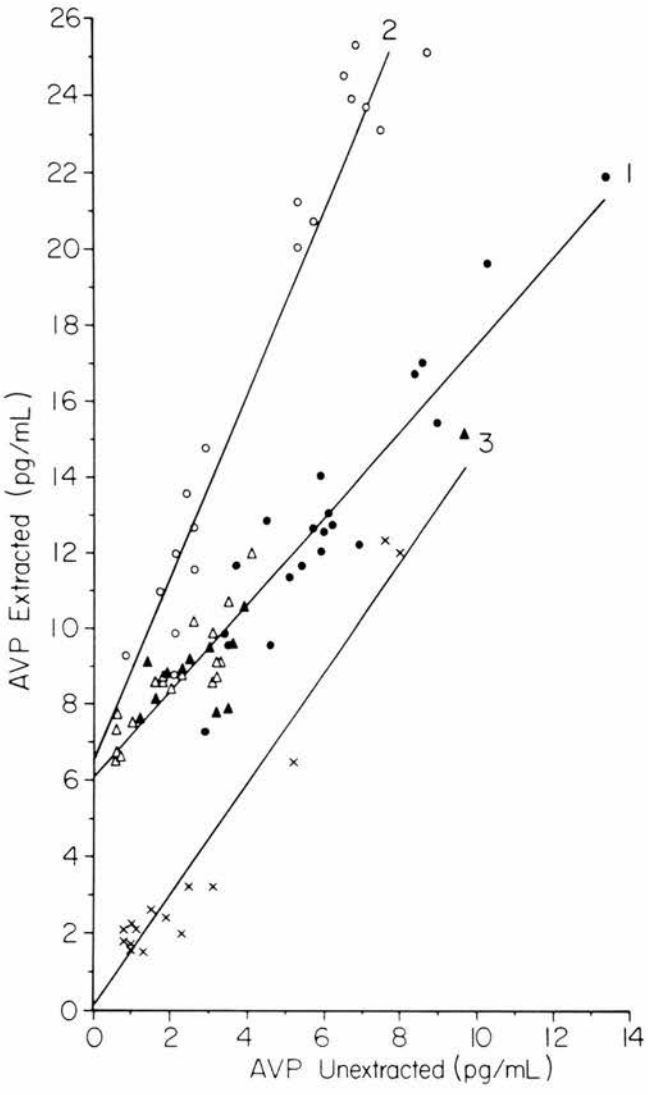

FIG. 1. Relationship between plasma AVP measured in unextracted plasma and in plasma after extraction in five dogs. Each symbol represents measurements in one dog. Regression line 1 is for three dogs in which there was a similar relationship. Regression lines 2 and 3 are for dogs in which the relationship was different. Regression equations are given in the text.

Experimental protocol

After the surgical procedures were completed a period of 1 h was allowed for stabilization of the animals. Each experiment lasted 160 min. At the end of each 10-min period urine was collected for measurement and analysis and a 1-min record of cardiovascular variables was made. At the midpoint of each 10-min period a 10-mL sample of arterial blood was taken for measurement of electrolytes, osmolality, and plasma AVP concentration. After three 10-min periods the balloon in the left atrium was distended so as to partially occlude the mitral orifice and raise left atrial pressure by about 15 mmHg. Left atrial distension was maintained for 90 min, the distension was then removed and samples were collected for a further four 10-min periods. To allow statistical analysis the control periods were taken to be the three 10-min periods before atrial distension and the final three 10-min periods. The experimental values were the average of the nine 10-min periods during atrial distension.

Values for the control and experimental periods were compared using a Student's *t*-test for paired data.

Results

The experiments were carried out on nine dogs. At the start of the experiments the average value (±SD) of the arterial pH was 7.41 ± 0.15, Pa_{CO_2} was 33.9 ± 3.3 mmHg and Pa_{O_2} was 153 ± 37 mmHg. Plasma sodium concentration (±SD) was 148 ± 10.6 mmol/L, plasma potassium concentration was 3.4 ± 0.35 mmol/L, and plasma osmolality was 292 ± 12.8 mosmol/kg. There were no significant changes in plasma composition throughout the experimental periods.

Inflation of a balloon in the left atrium to partially obstruct the mitral orifice caused an increase in mean atrial pressure (mean ± SEM) of 13.9 ± 0.78 mmHg, an increase in heart rate of 47 ± 13 beats/min, and a decrease in mean arterial pressure of 11 ± 5 mmHg. The time course of the cardiovascular changes is shown in Fig. 2. The increase in left atrial pressure was maintained throughout the 90-min period of mitral obstruction. Mean arterial pressure fell about 20mmHg when mitral obstruction began but gradually recovered through the 90-min period so that at the end of the 90 min the arterial pressure was 8 mmHg less than the control values. Heart rate remained elevated during atrial distension but showed a slow decline of about 20 beats/min during the 160-min experimental period.

The time courses of the average changes in urine flow and composition and plasma AVP (nonextracted) are shown in Fig. 3. After starting left atrial distension urine flow usually did not change until the second 10-min period and reached a maximum rate during the fifth 10-min period (range, third to ninth 10-min period) after the start of atrial distension. The increase in urine flow was always accompanied by a dilution of the urine with a consequent increase in free-water clearance.

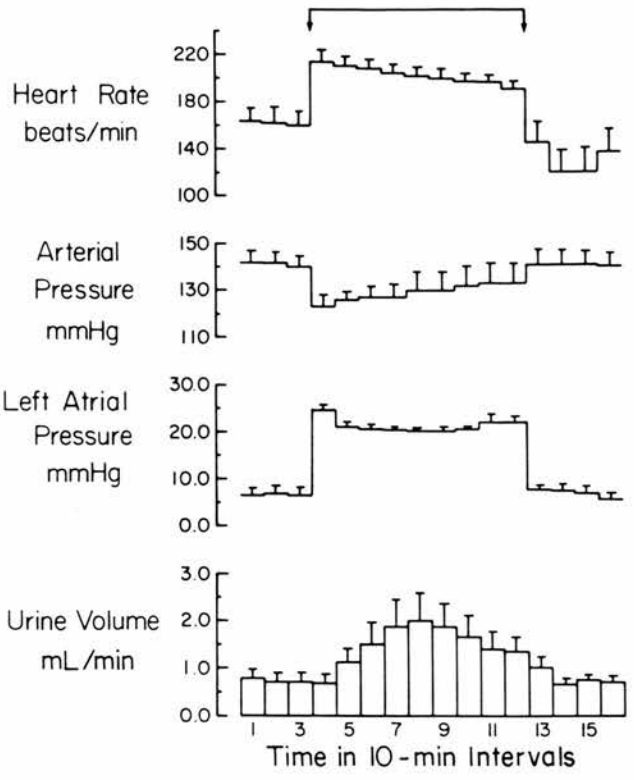

FIG. 2. Changes (mean ± SEM) in heart rate (beats per minute), arterial pressure (millimetres of Hg), left atrial pressure (millimetres of Hg), and urine volume (millilitres per minute) in nine experiments. Left atrial pressure was increased by partial obstruction of the mitral orifice for the 90 min indicated by the arrows.

Urine osmolality usually began to decrease during the second 10-min period after atrial distension and reached a minimum between the fourth and eighth 10-min periods. Free-water clearance began to increase in the second 10-min period and reached a maximum in the fifth 10-min period (range, fourth to eighth 10-min periods). After the peak change was reached urine flow, urine osmolality, and free-water clearance began to return to control values but complete return to control values did not occur until after release of atrial distension. Indeed it was not possible to show statistically that the changes in urine osmolality, free-water clearance, and urine volume were transient. Comparison, using a paired *t*-test, of the values after 50 min with the values in the last 10-min period of atrial distension showed no significant differences for any of the three variables. Linear regression analysis of the values in the last five 10-min periods during atrial distension failed to show a significant change of urine osmolality, free-water clearance, or urine volume during this time period. The values of these three variables in the last 10 min of the period of distension were all significantly different ($p < 0.05$) from the mean values of the post-

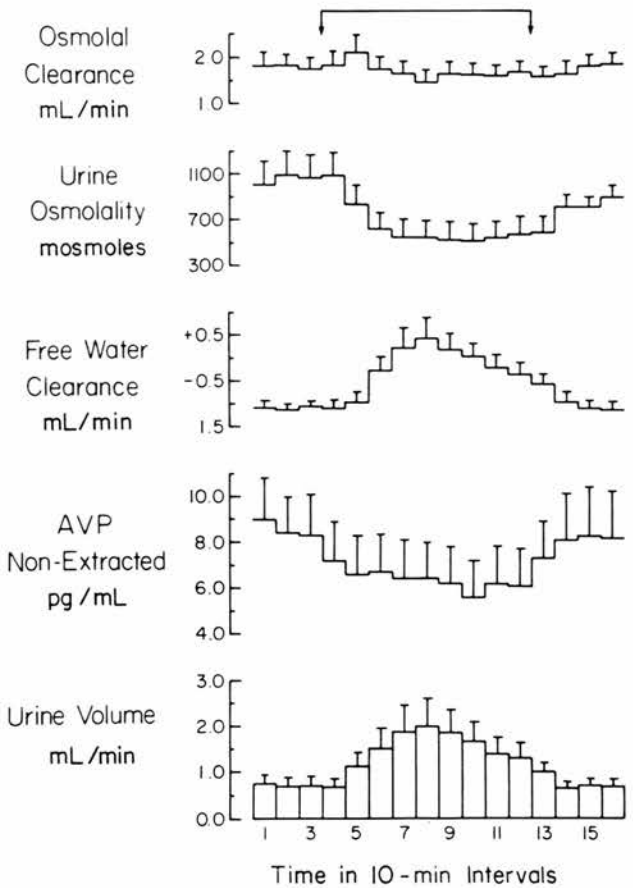

FIG. 3. Changes (mean ± SEM) in osmolal clearance (millilitres per minute), urine osmolality (milliosmoles per kilogram), free-water clearance (millilitres per minute), plasma AVP, nonextracted (picograms per millilitre), and urine volume (millilitres per minute). Conventions as in Fig. 2.

distension control period. Plasma AVP was usually decreased in the sample taken 5 min after the start of atrial distension and was close to its minimum value in the second 10-min period after the start of atrial distension. At this time AVP was significantly less than the predistension control values ($p < 0.01$). The lowered values of plasma AVP were maintained throughout the period of atrial distension. On release of the atrial distension plasma AVP was increased in the sample taken 5 min after release of the atrial distension and had reached a new steady level in the sample taken 15 min after release of the distension. There was a small increase in osmolal clearance at the start of atrial distension in five of the nine experiments, which reached a maximum rate in the second 10-min period and returned to control values in the third 10-min period. Other than these transient changes there were no changes in osmolal clearance throughout the experiment.

The values of the variables of urine flow, urine osmolality, free-water clearance, and plasma AVP in the 30-min control periods before left atrial distension were compared with the values in the 30-min control periods after left atrial distension. There were no significant differences between the values in the two control periods showing a complete return to predistension values after removal of the atrial distension. These values were combined to obtain a single average control value for comparison with experimental values (mean of the nine periods during atrial distension). Urine volume increased from a control value (mean ± SEM) of 0.7 ± 0.14 $mL \cdot min^{-1}$ to an experimental value of 1.58 ± 0.4 $mL \cdot min^{-1}$. Urine osmolality decreased from a control value (mean ± SEM) of 930 ± 126 $mosmol \cdot kg^{-1}$ to an experimental value of 594 ± 141 $mosmol \cdot kg^{-1}$. Free-water clearance increased from a control value (mean ± SEM) of −1.07 ± 0.12 $mL \cdot min^{-1}$ to an experimental value of −0.09 ± 0.25 $mL \cdot min^{-1}$. Plasma AVP (nonextracted) decreased from a control value (mean ± SEM) of 8.1 ± 0.15 $pg \cdot mL^{-1}$ to a mean experimental value of 6.2 ± 0.14 $pg \cdot mL^{-1}$. The experimental values for each of these variables were significantly different from the control values ($p < 0.01$). Examination of the relationship between urine osmolality and plasma AVP showed that in five of the nine experiments the minimum urine osmolality was less than plasma osmolality (mean minimum urine osmolality, ± SEM = 171 ± 24 $mosmol \cdot kg^{-1}$). In each of these five experiments plasma AVP (unextracted) was less than 6 $pg \cdot mL^{-1}$ and the mean value (±SEM) was 3.7 ± 0.8 $pg \cdot mL^{-1}$ at the time of minimal urine osmolality. Since the extracted values were never greater than the unextracted values this means that the extracted values would have been less than 6 $pg \cdot mL^{-1}$. In the other four experiments the minimum urine osmolality (mean ± SEM) was 691 ± 203 $mosmol \cdot kg^{-1}$ and the minimum plasma AVP (unextracted mean ± SEM) was 9.5 ± 3 $pg \cdot mL^{-1}$. In only one of these four experiments was plasma AVP less than 6 $pg \cdot mL^{-1}$.

The apparent transient nature of the mean changes in urine volume and free-water clearance shown in Fig. 3 was dependent upon the results in three experiments in which there was a high urine flow during the control periods (<1 $mL \cdot min^{-1}$). In these three experiments, an example of which is shown in Fig. 4, there was an obvious decline in free-water clearance and an increase in urine osmolality before removal of the atrial distension and at a time when plasma AVP was not changing.

Discussion

The results of the present investigation confirm that when atrial distension is maintained for 90 min increases in urine volume and free-water clearance and decreases in urine osmolality reach a maximum value

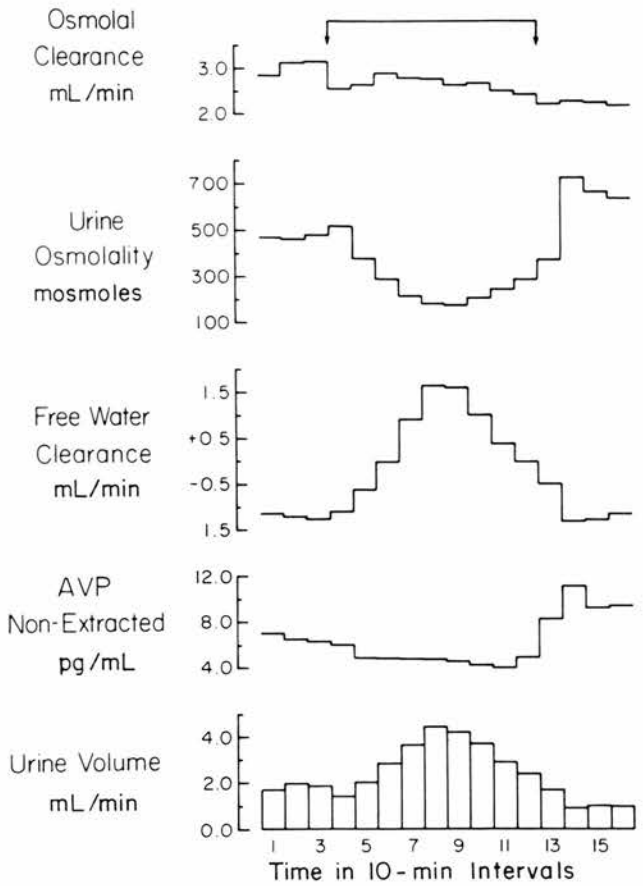

FIG. 4. Example of one experiment in which urine volume, free-water clearance, and osmolality began to return to control values during the period of atrial distension. Conventions as in Fig. 3.

and then, in some cases begin to return towards control values (Lawrence *et al.* 1973). However, complete return to control values was achieved only after release of the atrial distension. Previous investigations have all indicated a diuretic response with a maximum increase in urine flow occurring 20–50 min after the start of left atrial distension (Henry *et al.* 1956; Ledsome *et al.* 1961; Lydtin and Hamilton 1964; Lawrence *et al.* 1973).

The statistical significance of the time course of these changes has not been tested previously. The mean values of urine flow, urine osmolality, and free-water clearance throughout the period of atrial distension were significantly different from the values in the control periods but it was not possible to confirm statistically that there was a decline from the peak values at the end of the period of atrial distension. The values of each of these variables in the last 10-min period of atrial distension were significantly different ($p < 0.05$) from those in the following control period, these findings confirming the previous observation of Lawrence *et al.* (1973). Thus the response to atrial distension is maintained for at least 90 min and it cannot be concluded that the response, at least in the present experiments, is transient during this time. Nevertheless, in three of the nine experiments (Fig. 4) there was an obvious peak change which was reached at about 50 min after which there was a return towards control values before atrial distension was removed.

In previous reports of the effects of atrial distension on plasma AVP (de Torrente *et al.* 1976; Zucker *et al.* 1979), plasma AVP was measured by RIA after an extraction procedure had been carried out. It was not stated in those reports whether the results given had been corrected for loss during the extraction procedure. In both reports only a single sample of plasma was taken for measurement of AVP before, during, and after left atrial distension. The precise time of sampling was variable as was the duration of left atrial distension. Thus it was not possible to discern the time course of the changes in plasma AVP nor was it clear how representative the single sample was of the whole period of atrial distension. Both groups also showed increases in urine volume and decreases in osmolality associated with atrial distension. There was no attempt to correlate the urinary changes with changes in AVP in individual experiments. This information is of particular importance in the work of de Torrente *et al.* (1975) because the mean plasma AVP during atrial distension remained greater than the value of 10 $pg \cdot mL^{-1}$ shown to be associated with maximal urinary osmolality in the conscious dog (Weitzman and Fisher 1978). Our results leave little doubt that plasma AVP (unextracted) was reduced by left atrial distension and that this reduction was maintained for the whole 90-min distension period. Because there is a high correlation between the values measured on extracted and unextracted plasma, the measurements on the unextracted plasma can give a reliable indication of changes in plasma AVP. The results are in disagreement with the statement made by Shu'ayb *et al.* (1965) that blood ADH returned to the preinflation level after 30 to 40 min. These authors (Shu'ayb *et al.* 1965) also described a marked increase in blood ADH, after removal of atrial distension, to values well above predistension values. The example of an individual experiment shown here (Fig. 4) does show some suggestion of a "rebound," but the average results (Fig. 3) do not confirm such a change.

The finding that both the changes in plasma AVP and the increase in urine volume and free-water clearance and the decrease in urine osmolality were all maintained throughout the 90-min period of atrial distension is consistent with the hypothesis that the urinary changes are secondary to the changes in plasma AVP. Examination of the details of the time relationship between changes in plasma AVP and urine osmolality provides further support. At the start of atrial distension there was a

rapid (5–15 min) decrease in plasma AVP to a new steady level. The delay in reaching the maximum change in urine volume and osmolality of 40–50 min was similar to the time course of water diuresis following ingestion of water in the conscious dog (O'Connor 1962). On release of the atrial distension plasma AVP was usually increased 5 min after the end of atrial distension (Fig. 3). Urine osmolality and volume returned to control values with a 10-min delay after removal of atrial distension. The more rapid return to control values at the end of the period of atrial distension is consistent with the effects of intravenous injection or infusion of pituitary extract (O'Connor 1962). Thus the time courses of the onset of the diuretic response and the return to control values at the end of atrial distension are consistent with the observed changes in plasma AVP. Although it was not possible to show statistically that the diuretic response was usually transient there was no doubt that in some experiments (Fig. 4) the urinary changes declined before release of atrial distension. There was no evidence in any of these experiments that plasma AVP was also returning to control values. It is possible that the kidney may respond with a transient diuresis to a sustained decrease in plasma AVP. That this is a possibility has been demonstrated previously by Mason and Ledsome (1971). They showed that in hydrated anaesthetized dogs changing the rate of infusion of ADH from 0.4 $mU \cdot kg^{-1} \cdot min^{-1}$ to 0.04 $mU \cdot kg^{-1} \cdot min^{-1}$ was associated with a transient increase in urine volume and free-water clearance. At that time the lower dose of 0.04 $mU \cdot kg^{-1} \cdot min^{-1}$ was thought to be adequate to produce maximal urinary concentration. However, it is clear from the work of Weitzman and Fisher (1978) that in hydrated conscious dogs an infusion rate of about 0.13 $mU \cdot kg^{-1} \cdot min^{-1}$ is required to produce maximal urinary concentration. Thus a change in the rate of infusion of vasopressin from one steady level to another can be accompanied by changes in urine volume and osmolality which reach a maximum change and then return towards control values. The observation in some experiments that maximum urinary changes are reached and there may then be decline towards control values at a time when there is a maintained low value of plasma AVP, does not contradict the hypothesis that the diuretic response is due to the fall in plasma AVP. Further evidence to support this hypothesis would be provided if it could be shown that the relationship between urine osmolality and plasma AVP during the experiments was similar to that established in conscious dogs (Weitzman and Fisher 1978). The relationship would have to hold not only for the average results but also for the results of each individual experiment (Kappagoda *et al.* 1974). This relationship could not be examined in the present experiments because it was not possible to compare our results on unextracted plasma with those obtained by Weitzman and Fisher (1978) after extraction. Nevertheless, it was of interest to note that in those experiments in which the urine osmolality fell to less than plasma osmolality the plasma AVP (unextracted) was always less than 6 $pg \cdot mL^{-1}$. Since the extracted values are always less than the unextracted values this means that after extraction these values would have been less than 6 $pg \cdot mL^{-1}$. Further evidence that the diuretic response to left atrial distension is dependent upon a decrease in plasma AVP has been provided by experiments in which a continuous infusion of AVP has been given during atrial distension. Ledsome *et al.* (1961) and Lydtin and Hamilton (1964) gave infusions of vasopressin at a rate of 0.025 $mU \cdot kg^{-1} \cdot min^{-1}$ and reported that the diuretic response to atrial distension was not eliminated. At that time, this rate of infusion was considered to be adequate to cause maximal urine concentration. The effects of infusions of vasopressin were examined in more detail by Ledsome and Mason (1972). They found that infusion at a rate of 0.1 $mU \cdot kg^{-1} \cdot min^{-1}$ was needed to prevent any changes in urine volume during atrial distension and that infusion at rates greater than 0.1 $mU \cdot kg^{-1} \cdot min^{-1}$ was needed to prevent changes in urine osmolality. It was concluded from their experiments that infusion at a rate greater than 0.1 $mU \cdot kg^{-1} \cdot min^{-1}$ was needed to provide a maximally effective dose. These observations have been confirmed more recently by Weitzman and Fisher (1978) who found that infusion at a rate of 0.136 $mU \cdot kg^{-1} \cdot min^{-1}$ was needed to induce maximal urinary concentration in hydrated conscious dogs. This rate of infusion produced a plasma AVP concentration of 4.6 $\mu U \cdot mL^{-1}$ (~10 pg). Thus it is clear that if vasopressin is infused at a rate sufficient to induce maximal urinary concentration left atrial distension no longer induces an increase in urine volume or a decrease in urine osmolality. The small increase in osmolal clearance observed in the first 20–30 min of atrial distension (Ledsome and Mason 1972) is not affected by infusion of vasopressin and presumably depends upon a different mechanism. This small increase in osmolal clearance can be seen in Fig. 3 but did not reach statistical significance in the present series.

These observations do not prove that it is stimulation of left atrial receptors that causes a decrease in plasma AVP. Left atrial distension produced by partial mitral obstruction raises the pressure throughout the pulmonary vascular bed (Ledsome *et al.* 1961). There is, however, evidence that the diuretic response depends upon a stimulus arising from the left atrium (Henry *et al.* 1956). Generation of impulses in afferent myelinated fibres in the vagus nerves has been shown to be responsible for a reflex increase in heart rate (Kappagoda *et al.* 1974) and a decrease in efferent renal

nerve activity (Linden *et al.* 1980). The recent work of Kappagoda and Padsha (1981) indicates that during distension of the left atrium for a period of 60 min there is likely to be a diminution in activity arising from left atrial receptors. Other observations have been made over different time periods. Gilmore and Zucker (1974) were unable to show any adaptation over 15 min. Greenberg *et al.* (1973) showed decreased sensitivity of atrial receptors to changes in atrial pressure in dogs maintained in cardiac failure for periods of 1 week to 2 months. Linden *et al.* (1980) showed that the decrease in renal nerve activity induced by stimulation of atrial receptors was maintained for at least 30 min. More detailed examination of adaptation of atrial receptors to an increased stimulus will be needed to resolve this disagreement. The results of the present experiments do not support the hypothesis that the decrease in urine volume and free-water clearance seen in some dogs during atrial distension for 90 min is due to adaptation of the receptors to the stimulus because plasma AVP remained decreased throughout this period.

The results described are consistent with the hypothesis that left atrial distension causes stimulation of left atrial receptors and a decrease in the release of vasopressin from the neurohypophysis. The resulting decrease in plasma AVP is maintained for at least 90 min and is accompanied by changes in urine volume and osmolality which are consistent with their being caused by the decrease in plasma AVP. The increase in osmolal clearance which has previously been shown to occur in the first 20–30 min after atrial distension in anaesthetized dogs, and is independent of plasma AVP, is likely to be secondary to the decreases in efferent renal sympathetic nerve activity (Linden *et al.* 1980; Karim *et al.* 1972) and in renal vascular resistance (Mason and Ledsome 1974) associated with stimulation of left atrial receptors. These conclusions do not exclude the possibility of other agents acting on the kidney secondary to left atrial distension but there is evidence that the humoral agent acting on the kidney during left atrial distension is only diuretic (Linden and Sreeharan 1979) and not natriuretic. Positive proof that vasopressin is the humoral agent must await demonstration of a consistent relationship between plasma AVP and urine osmolality in individual experiments during stimulation of atrial receptors.

Acknowledgements

The authors are grateful for excellent assistance from Jeannie Sharp and David Morton.

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J. Physiol. (1983), **338**, *pp.* 413–421
With 2 text-figures
Printed in Great Britain

PLASMA VASOPRESSIN CONCENTRATION IN THE ANAESTHETIZED DOG BEFORE, DURING AND AFTER ATRIAL DISTENSION

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(*Received 15 April 1982*)

SUMMARY

1. Plasma vasopressin (AVP) concentration in dogs anaesthetized with chloralose was measured by radioimmunoassay and was within the range of 2–5 pg/ml. during control periods.

2. Distension of the left atrium led within 2 min to a fall in plasma AVP concentration which reached a steady lower value within 4 min.

3. After cessation of atrial distension the AVP concentration returned to pre-distension values within 4 min.

4. Cooling the cervical vagosympathetic nerves to 8–10 °C led to a rise in plasma AVP concentration.

5. Atrial distension during cooling of the vagi resulted in a further increase of plasma AVP concentration.

INTRODUCTION

Distension of the left atrium is associated with a diuresis in anaesthetized dogs (Henry, Gauer & Reeves, 1956). Measurements of plasma vasopressin concentration by bioassay have usually shown decreases in the concentration of vasopressin during atrial distension (Baïsset & Montastruc, 1959; Share, 1965; Shu'ayb, Moran & Zimmermann, 1965; Johnson, Moore & Segar, 1969; Brennan, Malvin, Jochim & Roberts, 1971). Kappagoda, Linden, Snow & Whitaker (1974) were unable to demonstrate such changes with the bioassay procedure they used. More recently measurements of plasma vasopressin concentration by radioimmunoassay (de Torrente, Robertson, McDonald & Schrier, 1975; Zucker, Share & Gilmore, 1979) have confirmed that plasma vasopressin decreases during atrial distension.

Previously published work does not provide a clear description of the time course of the changes in plasma vasopressin concentration which accompany left atrial distension. Shu'ayb *et al.* (1965) took up to twelve samples per hour but at different intervals in individual experiments. They described the changes in plasma vasopressin concentration as starting within 5 min of atrial distension and reaching a nadir in 15–20 min. After atrial distension was stopped there was an increase within 5 min. Other workers (Johnson *et al.* 1969; Zucker *et al.* 1979) took single samples before atrial distension, 20–25 min after starting distension and 20–25 min after stopping distension.

Until recently the half-life of vasopressin in the plasma of anaesthetized dogs was thought to be about 6 min (Share, 1962; Lauson, 1967). Studies by Weitzman & Fisher (1978) have shown that the disappearance of the hormone from the blood does not follow a simple exponential decay, but that it can be described by a two component system, one with a time constant indicating a half-life of 1·4 min, the other giving a half-life of 4·1 min. It is therefore possible that changes may occur earlier after an intervention than would be apparent when plasma is sampled infrequently. The present study was designed to examine the rate of change of plasma arginine vasopressin (AVP) at the start of atrial distension and immediately following the removal of atrial distension. It was possible to demonstrate that a new steady state of plasma AVP concentration was reached 4 min after the start and end of atrial distension. In the second part of the study the effects were examined of cold blockade of the vagus nerves on plasma AVP and on the changes in plasma AVP induced by atrial distension.

METHODS

Eight dogs weighing 16–28 kg were given morphine sulphate, 0·5 mg/kg, s.c. One hour later a saphenous vein was catheterized and 10 ml./kg of α-chloralose solution was infused, within 5 min, to induce anaesthesia (BDH Chemicals, U.K.; 1 g/100 ml. in 0·9 % (w/v) NaCl). As soon as possible thereafter positive pressure ventilation (Model 614, Harvard Apparatus Co., MA) with 40 % O_2 in N_2 was begun at a rate of 14 breaths/min and a tidal volume of 50 ml./4 kg body wt. When the chest was opened an exhalation valve (Ohio Chemical) provided an expiratory resistance of 3 cmH_2O.

Arterial pH, P_{CO_2} and P_{O_2} were measured with electrodes (Model 165/2, Corning Glass Works, MA) in blood samples of 3 ml. drawn at approximately hourly intervals from the femoral artery. The pH was maintained in the range 7·3–7·4 and arterial P_{CO_2} between 35 and 40 mmHg (1 mmHg = 0·13 kPa) by adjusting the tidal volume and by giving aliquots of $NaHCO_3$ (1 M) I.V. No adjustments were made after the experimental protocol was begun. During the surgical procedures 7·7 ml./kg of dextran (Dextran 70 in 0·9 % NaCl, Pharmacia Ltd., Quebec) was infused. After surgery was completed anaesthesia was maintained with a constant infusion of chloralose (0·5 g/100 ml. saline at 2 ml./min).

The chest was opened in the left fifth intercostal space and a balloon placed in the left atrium as described previously (Ledsome, Linden & O'Connor, 1961). The balloon was made from a 2 cm length of a surgical glove tied to a 2 mm diameter Teflon catheter. It was inserted into the atrium through the appendage and withdrawn so that the ligature securing the balloon to the catheter was close to the tip of the appendage. The catheter was clamped to prevent movement when the balloon was inflated. Femoral arterial pressure and left atrial pressure were recorded through 150 mm lengths of Teflon tubing (1 mm bore). To each cannula was attached a strain guage (P_{23}Db, Statham Inst. Co., Puerto Rico) and after d.c. amplification the pressure was recorded on an ultraviolet light recorder (Visicorder, 1608, Honeywell, Denver, CO). The frequency response of these systems tested by the method of Hansen (1949) was flat (±5 %) to better than 35 Hz. Mean pressures were obtained electrically. Heart rates were counted from an electrocardiogram over periods of 30 sec. The cervical vagosympathetic nerves were exposed and the nerve sheath removed over a 30 mm length of the nerve. The desheathed portion of each nerve was placed on a cooling thermode made of a silver-plated copper block 25 mm wide and attached to a thermo-electric cooling module (Mectron Ltd., Langley, Bucks). Temperature of the nerve was measured by thermistors placed in the grooves in the thermodes in which the nerves lay. Preliminary experiments showed that the temperature in the nerve was within 1 °C of the temperature indicated by the thermistors. The nerves and cooling modules were immersed in mineral oil. Oesophageal temperature was maintained at 37 ± 1 °C using a heated table and temperature controller (Yellow Springs Inst. Co., Yellow Springs, OH).

Experimental protocol. After the surgical procedures were completed 1 hr was allowed for stabilization. Then a sample of arterial blood (10 ml.) was taken and a recording made of arterial

pressure, left atrial pressure and heart rate. The blood removed was replaced with an equal volume of dextran. Sampling and recording were repeated at 2 min intervals. After three samples had been taken the balloon in the left atrium was inflated rapidly to raise left atrial pressure by about 12 mmHg. The appropriate inflation volume (10–20 ml.) was determined before the experimental period. Atrial distension was maintained for 10 min during which time five more blood samples were taken. The balloon was then deflated and sampling and recording were continued for a further 10 min (five samples). Both vagosympathetic nerves were cooled to less than 10 °C (mean, 9·2 °C ± 0·6, S.D.). After the nerves had been at that temperature for 10 min, a sample of blood and a recording were taken and the atrium was then distended by the same amount as previously. After 10 min, sampling was repeated and then the atrial distension was removed. After a further 10 min the sampling was repeated. The vagosympathetic nerves were rewarmed and after they had been at body temperature for 10 min, sampling and atrial distension was repeated at the same time intervals as before.

Comparison of values in the control periods and in the experimental periods during atrial distension was made using a Student's *t* test for paired data.

Radioimmunoassay. The radioimmunoassay (RIA) for plasma AVP was carried out as described earlier (Ledsome, Wilson & Ngsee, 1982) with the exception that all plasma samples underwent extraction prior to measurements. The percentage recovery after extraction was 86 ± 5·5 % (mean, ± S.D.). Values presented here were corrected for extraction losses.

Osmometry. 3 ml. of blood was mixed with two drops of heparin (heparin sodium, 1000 u./ml.) and centrifuged. Plasma osmolality was measured by freezing point depression (Osmette, Precision Systems). The average difference between duplicate estimations of osmolality was 2·6 m-osmole/kg.

RESULTS

The first test of atrial distension was used to examine the time course of the changes in plasma AVP concentration, atrial pressure, heart rate and left atrial pressure in eight dogs. The average results in seven dogs are shown in Fig. 1. Atrial distension caused an immediate increase in left atrial pressure and a rapid fall in arterial pressure. This reached its nadir about 19 sec later but quickly rose again and 2–4 min after the start of distension attained a constant value about 10 mmHg below the pre-distension value. Arterial pressure returned rapidly to its pre-distension value as soon as the distension was released. Heart rate increased immediately on atrial distension, by about 30 beats/min, and remained elevated while the atrium was distended.

Plasma AVP concentration was already decreased, compared to the average of the control values, in the sample taken 2 min after atrial distension and by 4 min after the start of distension had reached a still lower value which remained with little change until the end of the 10 min distension period. It then increased again and reached its pre-distension value within 4 min.

The AVP concentration in four of the five samples taken during atrial distension was significantly less ($P < 0·05$) than the average of the three pre-distension samples and the last four post-distension samples (Fig. 1). The average AVP concentration during atrial distension was significantly less ($P < 0·025$) than the average of the pre-distension and post-distension values.

In one of the eight dogs studied atrial pressure rose steeply to 58 mmHg when the atrium was first distended, and systemic arterial pressure fell by 46 mmHg. About 2 min later atrial pressure was reduced to 25 mmHg by removing saline from the balloon. The findings in this experiment are shown in Fig. 2. In contrast to the results in the other seven experiments there was a marked rise in plasma AVP concentration

present at 2 and 4 min after the start of atrial distension. After that time there was a gradual decline in plasma AVP concentration back to control values.

In the same dogs when the vagosympathetic nerves were cooled to 8–10 °C (9·2 ± 0·6, mean ± S.D.) there was a significant increase in plasma AVP concentration and a significant increase in mean arterial pressure but no significant change in heart rate (Table 1). When atrial distension was repeated with the vagus nerves cooled there

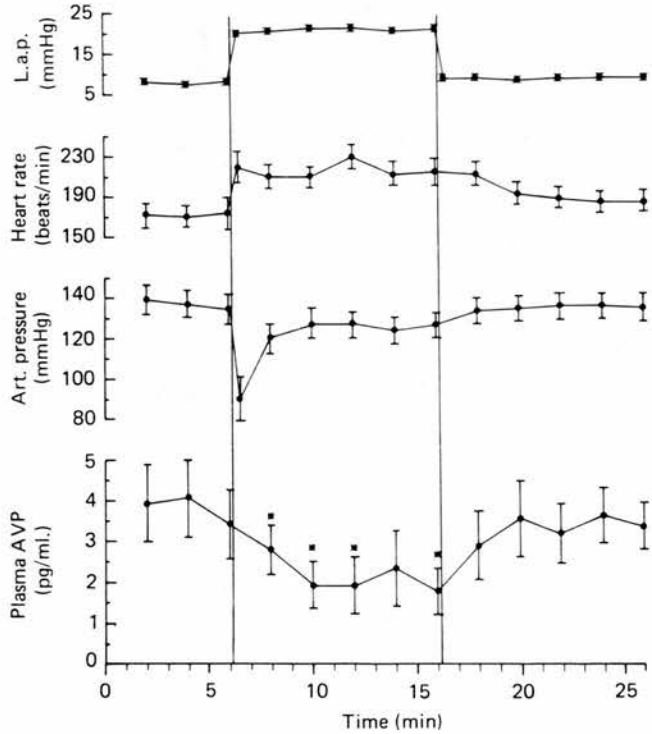

Fig. 1. Changes observed in response to left atrial distension during the period between the vertical lines. Each point represents the mean (+S.E. of mean) of results in seven dogs. Asterisks indicate that the plasma AVP value was less than the average plasma AVP during the control periods ($P < 0·05$). Plasma samples for AVP were taken at the times indicated by the plotted points. L.a.p. is left atrial pressure; art. pressure is mean femoral arterial pressure.

was a further significant increase in plasma AVP concentration in contrast to the decrease when the vagus nerves were at normal body temperature; a fall in arterial pressure not significantly different from that with the vagi warm and an increase in heart rate which was significantly smaller than with the vagi warm. Atrial distension was repeated with the vagi rewarmed. The changes in arterial pressure, heart rate and plasma AVP concentration were not significantly different from those during the first test of atrial distension. The statistical analysis of the differences in the results obtained with the vagosympathetic nerves warm or cold is presented in Table 1.

The plasma osmolality was 282 ± 11·1 m-osmole/kg (mean, ± S.D.) and there were no significant changes either during atrial distension or during the course of the experiments. There were no significant changes in plasma AVP concentration or arterial pressure between the control periods at the start and the end of the protocol.

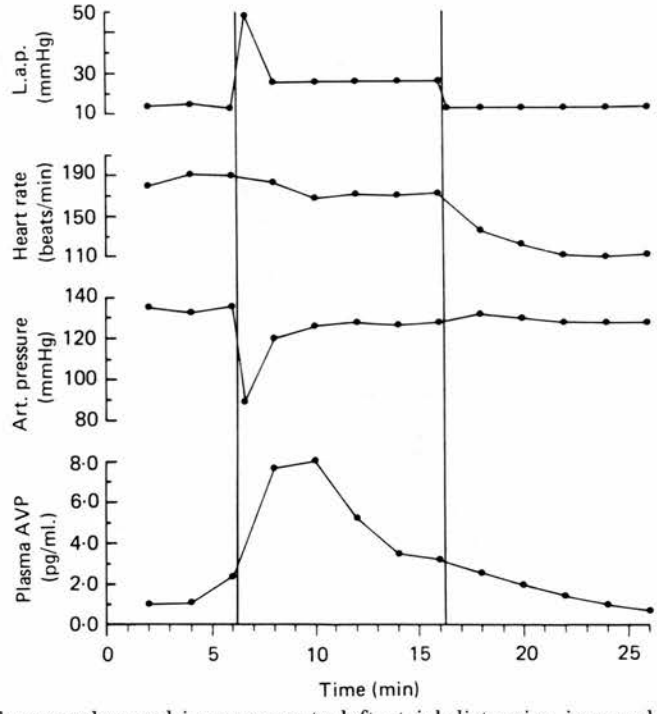

Fig. 2. Changes observed in response to left atrial distension in one dog in which atrial distension was excessive, for a brief period. Conventions as in Fig. 1.

TABLE 1. Plasma AVP concentration, mean arterial pressure and heart rate during control periods and during atrial distension with the vagi at normal body temperature or cooled to 8–10 °C

	Initial control	Atrial distension	Final control	$\bar{d}$	P
Vagi intact					
Plasma AVP (pg/ml.)	3·2±0·7	2·5±1·1	3·1±1·5	−0·54	< 0·05
Art. pressure (mmHg)	132±6·5	125±5·4	133±6·1	−7·6	< 0·01
Heart rate (beats/min)	175±14	205±13	172±12	+32	< 0·05
Vagi cool					
Plasma AVP (pg/ml.)	5·0±1·1	8·1±1·2	6·0±1·8	+2·6	< 0·05
Art. pressure (mmHg)	146±5·5	140±6·2	145±5·6	−5·7	< 0·05
Heart rate (beats/min)	170±9·0	177±12	158±12	+13	< 0·05
Vagi intact					
Plasma AVP (pg/ml.)	2·8±0·5	1·5±0·4	2·7±0·4	−1·2	< 0·05
Art. pressure (mmHg)	133±4·8	123±5·2	131±4·3	−9·3	< 0·05
Heart rate (beats/min)	157±12	203±8·3	178±9·6	+36	< 0·05

Change during vagal cooling	Average control $\bar{d}$	Average control P	Atrial distension $\bar{d}$	Atrial distension P
Plasma AVP (pg/ml.)	+2·6	< 0·05	+6·1	< 0·05
Art. pressure (mmHg)	+13·1	< 0·01	+15·8	< 0·01
Heart rate (beats/min)	−6	n.s.	−27	< 0·05

Results from eight dogs. For comparison of values during atrial distension with control values the average of the initial and final control values was used ($\bar{d}$ = mean difference). For comparison of values with the vagi at body temperature and cooled, the averages of the two control values with the vagi cool were compared to the averages of the four control periods with the vagi intact. The values during atrial distension with the vagi cool were compared with the averages of the two periods of atrial distension with the vagi intact.

DISCUSSION

The results confirm the observations of other authors that atrial distension caused by inflation of a balloon in the left atrium leads to a decrease in plasma AVP concentration (Baïsset & Montastruc, 1959; Shu'ayb *et al.* 1965; Johnson *et al.* 1969; Brennan *et al.* 1971; de Torrente *et al.* 1975; Zucker *et al.* 1979) but differ from those of previous investigators in that changes in plasma AVP were measured over a much shorter time. Also the changes which were measured occurred within a lower range of plasma AVP concentrations. The rate of disappearance from the plasma inferred from our results would be consistent with the data of Weitzman & Fisher (1978), showing a rapid component of the plasma clearance of AVP. The fact that plasma AVP concentration had already fallen after 2 min of atrial distension shows the rapidity with which changes in plasma AVP can occur; caution should therefore be used in regarding the AVP concentration in single samples of plasma as the average AVP value over a period of time, e.g. of an entire period of atrial distension lasting 30 or 50 min. Our results suggest that plasma AVP usually reaches a new steady level 4 min after the start of atrial distension and they also show that rapid changes in plasma AVP may occur. We followed changes in plasma AVP concentration for only 10 min. It cannot be assumed that plasma AVP concentration would then remain constant for a long period of atrial distension. When atrial distension is prolonged the diuretic response reaches a peak after 40–50 min and then declines (Henry *et al.* 1956; Lydtin & Hamilton, 1964; Lawrence, Ledsome & Mason, 1973). Shu'ayb *et al.* (1965) claimed that both ADH and urinary flow started to return to the pre-inflation level in 30–40 min, although this statement was not borne out in all their illustrations.

Carotid occlusion has been shown to lead to a marked elevation of plasma AVP in anaesthetized dogs (Share, 1965), and at least a transient increase in plasma AVP would have been expected because atrial distension was associated with immediate profound fall of systemic arterial pressure. Indeed, on one occasion when the degree of atrial distension was greater than intended and there was a large decrease in arterial pressure there was also a marked transient increase in plasma AVP concentration (Fig. 2). Our observations illustrate that the effects of atrial distension depend upon the balance between the degree of atrial distension and the associated decrease in arterial pressure as was demonstrated by Thames & Schmid (1981).

The majority of previous investigators examining the effects of atrial distension on plasma AVP in anaesthetized dogs (Baïsset & Montastruc, 1959; Share, 1965; Shu'ayb *et al.* 1965; Johnson *et al.* 1969; Brennan *et al.* 1971; de Torrente *et al.* 1975) found pre-distension plasma AVP concentrations to be higher than the range of 1·5–6 pg/ml. (Bie, 1980) reported in 'normal' conditions in well hydrated, unanaesthetized, unstressed dogs and other mammals. That changes in plasma AVP over a similar range are associated with changes in urine osmolality in anaesthetized dogs was clearly shown by Weitzmann & Fisher (1978) who found that with infusion of AVP urine osmolality increased to plateau values at a plasma AVP concentration of 10 pg/ml. In our experiments the plasma AVP concentration was less than 10 pg/ml. during both the control and experimental periods. Differences in the plasma AVP concentration in different series are likely to be due to the use of different anaesthetic agents, the degree of hydration and the amount of blood loss. Chloralose

anaesthesia, by itself, does not lead to an increase in plasma AVP (Ginsberg & Brown, 1956) and surgical trauma, not associated with blood loss, does not necessarily cause an increase in plasma AVP (Crone, Wilson, Ngsee, Turnbull & Leighton, 1982; Abed, Forsling, Nashat & Please, 1982). We were careful to minimize blood loss during surgery and our animals received a continuous infusion of saline during the experiment. Also the twenty-one blood samples taken were immediately replaced with an equal volume of dextran. It was of interest to note that base line plasma AVP did not increase during the experiments and that there were no progressive changes in arterial pressure, heart rate and atrial pressure in the same period.

Cooling of the vagi to between 8 and 12 °C prevents the diuretic response to left atrial distension (Henry & Pearce, 1956). At a temperature of 8–12 °C increases in the frequency of discharge, associated with atrial distension, in myelinated and some non-myelinated afferent fibres are blocked (Kappagoda, Linden & Sivanathan, 1979); and some vagal efferent fibres may also be blocked. It was therefore of interest to discover that vagal cooling by itself led to a significant rise of plasma AVP concentration (Table 1). This could have been due to loss of conduction in afferents from atrial stretch receptors, or from aortic baroreceptors or both. There is at present no direct evidence that aortic baroreceptors, like carotid baroreceptors (Share, 1965), influence the release of AVP. Arterial pressure rose when the vagus nerves were cooled, probably owing to interruption of aortic baroreceptor afferents. This in turn should have tended to diminish plasma AVP concentration, according to Share (1965) and Thames & Schmid (1981), by its effect on carotid baroreceptors. However, plasma AVP concentration increased and the increase was even greater when the left atrium was distended during vagal cold block. The decrease in systemic arterial pressure, in consequence of the mechanical reduction of cardiac output during atrial distension was as great with the vagi intact as it was when the vagi were cooled (Table 1). This demonstrated that it was not the systemic arterial pressure which, by affecting carotid baroreceptor stimulation, determined whether plasma AVP concentration during atrial distension would fall or rise; and that normal conduction in vagal afferent fibres was an essential condition for the observed reduction of AVP concentration during left atrial distension. This conclusion is supported by the recent work of Thames & Schmid (1981) who reported that during vagal cold block to 0 °C plasma AVP increased, in dogs with prior aortic baroreceptor deafferentation and with carotid sinus pressure held constant. The fact that the increase in heart rate associated with atrial distension was significantly less with the vagi cool than with the vagi intact suggests that a significant part of the increase in heart rate depends upon vagal afferents rather than on the fall in arterial pressure acting through carotid arterial baroreceptors. There is evidence that stimulation of atrial receptors causes a reflex increase in heart rate (Kidd, Ledsome & Linden, 1978). It is unlikely that blockage of vagal efferent fibres contributed to the difference because control heart rate did not change on vagal cooling.

Our results cannot prove that it is stimulation of atrial receptors that leads to a decrease in plasma AVP. They also provide no information regarding the relationship between the changes in plasma AVP and the diuretic response to left atrial distension, but the results are consistent with the hypothesis that atrial distension leads to a decrease in plasma AVP leading in turn to a water diuresis. The rise in plasma AVP

concentration observed during vagal cooling and the further rise during atrial distension, together with the renal vasoconstriction caused by atrial distension after vagotomy (Mason & Ledsome, 1974), explain the failure of atrial distension to produce a diuretic response after vagotomy.

This work was supported by the Medical Research Council of Canada and the B.C. Heart Foundation. The authors are grateful to Jeannie Sharp and David Morton for their excellent assistance.

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Distension of the pulmonary vein – atrial junctions and plasma vasopressin in the chloralose-anaesthetized dog[1]

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Received December 16, 1982

WILSON, N., and J. R. LEDSOME. 1983. Distension of the pulmonary vein – atrial junctions and plasma vasopressin in the chloralose-anaesthetized dog. Can. J. Physiol. Pharmacol. **61**: 905–910.

The effects of localized distension of the pulmonary vein – left atrial junctions on plasma arginine vasopressin (AVP) have been examined in chloralose anaesthetized dogs. Pulmonary vein distension caused an increase in heart rate and a decrease in plasma AVP concentration. Cooling the vagosympathetic nerves to 10°C caused an increase in arterial pressure and plasma AVP concentration and prevented the changes in heart rate and plasma AVP concentration caused by pulmonary vein distension. Cooling the vagus nerves to 16°C did not change heart rate, arterial pressure, or plasma AVP concentration but significantly reduced the changes in heart rate and plasma AVP concentration caused by pulmonary vein distension. Propranolol (0.5 mg/kg) decreased heart rate and prevented the increase in heart rate associated with pulmonary vein distension but did not abolish the decrease in plasma AVP concentration. It is concluded that distension of the pulmonary vein – left atrial junctions causes a decrease in plasma AVP concentration by stimulating atrial receptors with myelinated afferent fibres. The decrease in plasma AVP concentration is not secondary to the reflex changes in heart rate caused by pulmonary vein distension.

WILSON, N., et J. R. LEDSOME. 1983. Distension of the pulmonary vein – atrial junctions and plasma vasopressin in the chloralose-anaesthetized dog. Can. J. Physiol. Pharmacol. **61**: 905–910.

On a examiné, chez des chiens anesthésiés au chloralose, les effets d'une distension localisée de jonctions auriculaires de la veine pulmonaire inférieure gauche sur l'argipressine (AVP) plasmatique. La distension de la veine pulmonaire fit augmenter la fréquence cardiaque et diminuer la concentration d'AVP plasmatique. Le refroidissement des nerfs vagosympathiques à 10°C fit augmenter la pression artérielle et la concentration d'AVP plasmatique et il inhiba les variations de fréquence cardiaque et de concentration d'AVP plasmatique induites par la distension de la veine pulmonaire. Le refroidissement des nerfs vagues à 16°C ne modifia ni la fréquence cardiaque, ni la pression artérielle, ni la concentration d'AVP plasmatique; toutefois, il réduisit significativement les variations de fréquence cardiaque et de concentration d'AVP plasmatique provoquées par la distension de la veine pulmonaire. Le propranolol (0.5 mg/kg) diminua la fréquence cardiaque et prévint l'augmentation de fréquence cardiaque associée à la distension de la veine pulmonaire; il n'empêcha toutefois pas la diminution de la concentration d'AVP plasmatique. On conclut que la distension des jonctions auriculaires de la veine pulmonaire provoque une diminution de la concentration d'AVP plasmatique en stimulant les récepteurs auriculaires via des fibres afférentes myélinisées. La diminution de la concentration d'AVP plasmatique ne résulte pas des variations réflexes de la fréquence cardiaque induites par la distension de la veine pulmonaire.

[Traduit par le journal]

Introduction

Distension of the left atrium, caused by partial obstruction of the mitral orifice, results in a diuresis in anaesthetized dogs (Henry *et al.* 1956). This observation formed the basis for a hypothesis that stimulation of atrial receptors causes inhibition of the release of antidiuretic hormone from the neurohypophysis (Gauer and Henry 1976). However, the stimulus provided by mitral obstruction is not limited to the left atrium, because the pressure is raised throughout the pulmonary vascular bed and there are changes in heart rate (Ledsome *et al.* 1961). Henry *et al.* (1956) showed that obstruction of the pulmonary veins or embolization of the pulmonary vascular bed did not cause a diuresis. More recently, Schultz *et al.* (1982) have shown that left atrial distension, in conscious dogs, is accompanied by a decrease in plasma arginine vasopressin (AVP) concentration and plasma renin activity (PRA), but that partial occlusion of the pulmonary veins and pulmonary arteries does not cause hormonal changes. These experiments indicate that it is atrial distension rather than distension of the pulmonary vascular bed that is essential for the renal and hormonal effects of mitral obstruction.

The left atrial receptors lie in the endocardium close to the junctions of the pulmonary veins with the left atrium (Coleridge *et al.* 1957). Inflation of small balloons placed in the left pulmonary veins at their junctions with the left atrium has been shown to induce an increase in action potential activity from left atrial receptors. The increase in activity has many of the characteristics of the activity evoked by a more physiological stimulus, such as blood volume expansion (Kidd *et al.* 1978). We have used pulmonary vein dis-

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tension to examine the effects of localized stimulation of left atrial receptors on plasma AVP concentration. Because there are both myelinated and nonmyelinated afferent fibres arising from the left atrium we have used the technique of cooling the cervical vagosympathetic nerves (Kappagoda *et al.* 1979) to examine the afferent pathway of responses. Pulmonary vein distension causes a reflex increase in heart rate (Ledsome and Linden 1964), which may have influenced other vascular receptors; therefore, in some experiments, the reflex increase in heart rate was blocked with propranolol.

Methods

Eleven mongrel dogs weighing 14–26 kg were given morphine sulphate, 0.5 mg/kg, s.c. One hour later a saphenous vein was catheterized and 10 mL/kg of α-chloralose solution was infused, within 5 min, to induce anaesthesia (BDH Chemicals, U.K.; 1 g/100 mL in 0.9% w/v NaCl). As soon as possible thereafter, positive pressure ventilation (model 614, Harvard Apparatus Co., MA) with 40% O_2 in N_2 was begun at a rate of 14 breaths/min and a tidal volume of 50 mL/4 kg body wt. When the chest was opened an exhalation valve (Ohio Chemical) provided an expiratory resistance of 3 cmH_2O.

Arterial pH, P_{CO_2}, and P_{O_2} were measured with electrodes (model 165/2, Corning Glass Works, MA) in blood samples of 3 mL drawn at approximately hourly intervals from the femoral artery. The pH was maintained in the range 7.3–7.4 and arterial P_{CO_2} between 35 and 40 mmHg (1 mmHg = 0.13 kPa) by adjusting the tidal volume and by giving aliquots of $NaHCO_3$ (1 *M*) i.v. No adjustments were made after the experimental protocol was begun. During the surgical procedures 7.7 mL/kg of dextran (dextran 70 in 0.9% NaCl, Pharmacia Ltd., Quebec) was infused. After surgery was completed anaesthesia was maintained with a constant infusion of chloralose (0.5 g/100 mL saline at 2 mL/min).

Thoracotomy was performed through the fifth left intercostal space. The left lung was retracted and small balloons placed in each of three pulmonary veins, after the manner of Ledsome and Linden (1964). The left lung root was then tied tightly. Femoral arterial pressure and, in some experiments, left atrial pressure were recorded through 150-mm lengths of teflon tubing (1-mm bore) inserted into the femoral artery or the left atrial appendage. To each cannula was attached a strain gauge (P_{23}Db, Statham Inst. Co., Puerto Rico) and after direct current amplification the pressure was recorded on an ultraviolet light recorder (Visicorder, 1608, Honeywell, Denver, CO). Mean pressures were obtained electrically. Heart rates were counted from an electrocardiogram over periods of 30 s. The cervical vagosympathetic nerves were exposed and the nerve sheaths removed over a 30-mm length of the nerve. The desheathed portion of each nerve was placed on a cooling thermode made of a silver-plated copper block 25 mm wide and attached to a thermoelectric cooling module (Mectron Ltd., Langley, Bucks, U.K.). Temperature of the nerve was measured by thermistors placed in the grooves in the thermodes in which the nerves lay. Preliminary experiments showed that the temperature in the nerve was within 1°C of the temperature indicated by the thermistors. The nerves and cooling modules were immersed in mineral oil. Oesophageal temperature was maintained at 38 ± 1°C using a heated table and temperature controller (Yellow Springs Instrument Co., Yellow Springs, OH).

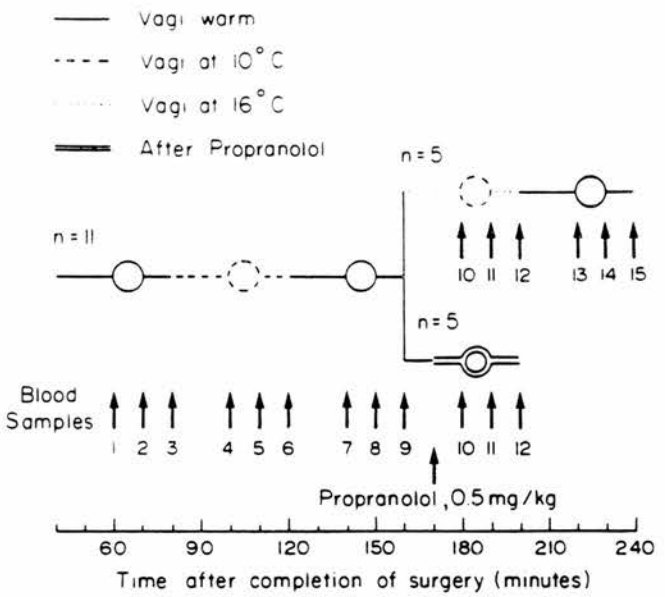

FIG. 1. Diagram of the experimental protocol. The times during which the pulmonary vein – atrial junctions were distended are indicated by the circles. Blood samples were taken at the times indicated by the arrows.

Experimental protocol

The general plan of the protocol is shown in Fig. 1. After the surgical procedures were completed 1 h was allowed for stabilization. During this time acid–base balance was corrected. When the experiment commenced a sample of arterial blood (10 mL) was taken into cold EDTA tubes. A recording was made of arterial pressure, heart rate and, in some experiments, left atrial pressure. The blood removed was replaced with an equal volume of dextran. The blood sample was immediately centrifuged at 4°C and the plasma separated. The red blood cells so obtained were suspended in dextran and the mixture used to replace the volume of subsequent blood samples. The three balloons in the pulmonary veins were then distended by injection of 1.0 or 1.5 mL of warm saline into each balloon (1.0 mL in dogs < 20 kg, 1.5 mL in dogs > 20 kg). After 10 min a second blood sample was taken and a recording made. The balloons were deflated and 10 min later a third blood sample was taken and a recording made. Both vagus nerves were then cooled to a temperature close to 10°C. It took 5 min to cool the nerves and they were maintained at 10°C for 15 min before the next test began. The protocol of blood sampling and recording, before, during, and after pulmonary vein distension was repeated. Cooling of the vagus nerves was stopped and a period of 20 min allowed for rewarming of the nerves. Sampling of blood and recording, before, during, and after pulmonary vein distension was repeated as before.

In five animals the vagus nerves were then cooled to 16°C and the test of pulmonary vein distension repeated as before. A final test of pulmonary vein distension was made with the

vagus nerves rewarmed. In another five animals, after the first three tests had been completed, propranolol (0.5 mg/kg) was injected intravenously. Ten minutes later a blood sample was taken, a recording made and the test of pulmonary vein distension was repeated as before.

Comparison was made between the values in the control periods before and after each test of pulmonary vein distension and the experimental values during distension of the pulmonary veins, using a Student's *t*-test for paired data. Comparison was also made between the values in the control periods before and after each period of vagal cooling and the values in the control periods during vagal cooling. Similar comparison was made between the values in the control periods before and after giving propranolol and between experimental values before and after giving propranolol.

Radioimmunoassay

The radioimmunoassay (RIA) for plasma AVP was carried out as described earlier (Ledsome *et al.* 1982*a*) with the exception that all plasma samples underwent extraction prior to measurements.

For this series of experiments, the extraction recovery was 87 ± 3% (n = 10). The results are corrected for extraction losses. The limit of detection, defined as 80% of the maximum binding was 0.27 ± 0.02 pg AVP (mean ± SE). 50% of the maximum binding was at 0.83 ± 0.03 pg AVP (both n = 10).

Results

At the start of the experiments the average value (±SD) of the arterial pH was 7.37 ± 0.05, P_{aCO_2} was 41 ± 6 mmHg and P_{aO_2} was 128 ± 40 mmHg. Plasma sodium concentration was 135 ± 8.9 mmol/L, plasma potassium concentration was 3.7 ± 0.3 mmol/L, and plasma osmolality was 284 ± 7.9 mosmol/kg. There were no significant changes in the above variables during the experimental period.

Distension of the pulmonary vein − left atrial junctions caused an increase in heart rate, a decrease in plasma AVP concentration, and a small decrease in mean arterial pressure. The statistical significance of these changes, when tested before cooling the vagus nerves and after rewarming the vagus nerves, is given in Table 1. When the vagus nerves were cooled to 10°C (9.3°C ± 0.6, mean ± SD) there was an increase in plasma AVP concentration, an increase in mean arterial pressure, and an increase in heart rate. Distension of the pulmonary vein − left atrial junctions then had no effect on plasma AVP concentration, arterial pressure, or heart rate (Table 1).

The tests of distension of the pulmonary vein − left atrial junctions were repeated in five of the same animals before and after the vagus nerves were cooled to 16°C (16.2 ± 1.2, mean ± SD). The results are given in Table 2. Cooling the vagus nerves to 16°C had no effect on the control values of plasma AVP concentration, mean arterial pressure, or heart rate. During distension of the pulmonary vein − left atrial junctions there was only a small and not significant decrease in plasma AVP concentration. The increase in heart rate which occurred during pulmonary vein distension with the vagus nerves at 16°C was significantly less than when the vagus nerves were warm.

The effect of distension of the pulmonary vein − left atrial junctions was also tested in five dogs before and after administration of propranolol (0.5 mg/kg, i.v.). Propranolol causes a significant decrease in heart rate but no change in mean arterial pressure or plasma AVP concentration (Table 3). In four dogs, left atrial pressure increased by an average of 1.8 cmH_2O (range 1−2.5). During pulmonary vein distension there was a significant decrease in plasma AVP concentration; plasma AVP concentration fell to a value that was significantly less than that during pulmonary vein distension before propranolol (Table 3). After giving propranolol, pulmonary vein distension no longer caused a significant increase in heart rate.

Discussion

Our results show that distension of the pulmonary vein − left atrial junctions causes a decrease in plasma AVP concentration, in addition to the increase in heart rate previously described (Ledsome and Linden 1964). This conclusion is based on measurements of single samples taken 10 min after an intervention. Previous investigators have sampled blood for AVP measurements a longer time after a cardiovascular intervention (Johnson *et al.* 1967; Schultz *et al.* 1982). Recent observations suggest that the half-life of AVP in dogs is 1.5 min (Chawlbinska-Moneta 1979) or can be described by a two component system one with a time constant indicating a half-life of 1.4 min, the other giving a half-life of 4.1 min (Weitzman and Fisher 1978). We have shown that during atrial distension induced by mitral obstruction a nadir in plasma AVP concentration is reached in 4 min and there is then little change up to 10 min after the start of the distension (Ledsome *et al.* 1982*b*). Thus samples taken at 10 min after an intervention are likely to be representative of the steady-state change induced in plasma AVP concentration.

In water-loaded anaesthetized dogs infused with AVP urine osmolality increases to plateau values at a plasma AVP concentration of 10 pg/mL (Weitzman and Fisher 1978). In previous reports of the effects of atrial distension plasma AVP concentration has been higher than this value (Shu'ayb *et al.* 1965; de Torrente *et al.* 1975) causing doubts about the ability of the changes in plasma AVP to lead to changes in urine concentration. In our experiments plasma AVP concentration was less than 10 pg/mL. Differences in plasma AVP concentration in different series are likely to be due to the use of different anaesthetic agents, the degree

TABLE 1. Effects of distension of the pulmonary vein – atrial junctions with the vagus nerves warm and cooled to 8–10°C. Values are means (± SEM) of results in 11 dogs. AVP, plasma vasopressin concentration; BP, mean femoral arterial pressure; HR, heart rate. The average difference between the control values and the values during distension is represented by $\bar{d}$

	Control	Distension	Control	$\bar{d}$	P
Vagi (warm)					
AVP, pg/mL	5.2±1.2	3.9±0.9	4.4±1.0	−0.9	< 0.05
BP, mmHg	139±3.8	134±4.2	136±2.4	−3.4	< 0.05
HR, beats/min	163±7.8	176±8.8	163±8.7	+12.4	< 0.01
Vagi (8–10°C)					
AVP, pg/mL	7.7±1.7*	8.2±1.8*	8.7±2.5*	0*	NS
BP, mmHg	146±3.8*	144±3.3*	142±2.7*	−0.6	NS
HR, beats/min	178±8.8*	171±5.8	171±6.2*	−3.6*	NS
Vagi (warm)					
AVP, pg/mL	6.0±1.5	4.5±1.2	6.9±2.2	−2.0	< 0.05
BP, mmHg	131±2.8	129±3.8	132±3.0	−2.8	NS
HR, beats/min	157±6.2	171±8.5	156±6.3	+14.3	< 0.01

NOTE: NS, not significant.
*Significantly different ($p < 0.05$) from average values with vagi warm.

TABLE 2. Effects of distension of the pulmonary vein – atrial junctions with the vagus nerves warm and cooled to 16°C. Values are means (± SEM) of results in five dogs. Conventions as in Table 1

	Control	Distension	Control	$\bar{d}$	P
Vagi (warm)					
AVP, pg/mL	4.2±0.2	2.7±0.1	4.3±0.7	−1.6	< 0.05
BP, mmHg	132±6	132±6	134±6	−2	NS
HR, beats/min	161±11	178±16	156±11	+20	< 0.05
Vagi (cool, 16°C)					
AVP, pg/mL	5.7±1.3	5.4±1.1*	5.7±1.4	−0.3	NS
BP, mmHg	135±6	133±4	137±8	−3.2	NS
HR, beats/min	167±9	177±8	172±8	+7*	< 0.05
Vagi (warm)					
AVP, pg/mL	4.7±0.7	3.4±0.8	3.7±0.7	−0.9	NS
BP, mmHg	133±8	127±8	123±10	−1	NS
HR, beats/min	175±9	195±16	187±11	+14	< 0.05

*Significantly different from the average values with vagi warm.

of hydration and the amount of blood loss. Chloralose anaesthesia, by itself, does not lead to an increase in plasma AVP concentration (Ginberg and Brown 1956) and surgical trauma, not associated with blood loss, does not necessarily cause an increase in plasma AVP concentration (Crone *et al.* 1982). We were careful to minimise blood loss during surgery: blood removed for analyses was replaced by reinfusing red blood cells and dextran, and our animals received a continuous infusion of saline. Inspection of the results in Tables 1 and 3 suggests there may have been a gradual increase in plasma AVP concentration during our experiments but it was not possible to show a statistical difference between the plasma AVP concentrations during the control periods, at the start and the end of the experiments. The difference in the control values of plasma AVP in the five experiments in Table 2 and the five experiments in Table 3 was a chance occurrence since animals were assigned at random to one or the other protocol. No attempt was made to relate the observed changes in the AVP concentration to urinary excretion in our experiments.

It has been shown by Kappagoda *et al.* (1979) that the increase in impulse activity in myelinated afferent fibres from atrial receptors, caused by pulmonary vein distension, was completely blocked when the vago-sympathetic nerves were cooled to 8–12°C. The increase in impulse activity in nonmyelinated fibres was not blocked unless the temperature was <8°C. We found that cooling the vagus nerves to 10°C caused an increase in arterial pressure, heart rate, and plasma AVP concentration. The increases in arterial pressure

TABLE 3. Effects of distension of the pulmonary vein − atrial junctions before and after administration of propranolol (0.5 mg/kg). Values are means (± SEM) of results in five dogs. Conventions as in Table 1

	Control	Distension	Control	$\bar{d}$	P
Before propranolol					
AVP, pg/mL	8.0±3.1	6.6±2.4	10.1±4.5	−2.5	< 0.05
BP, mmHg	130±1.8	126±5.8	129±3.4	−4.2	NS
HR, beats/min	155±8.8	167±9.0	159±9.5	+10.4	< 0.05
After propranolol					
AVP, pg/mL	8.7±3.3	4.8±1.5*	6.9±1.8	−3.0	< 0.05
BP, mmHg	128±3.6	126±4.1	133±4.3	−4.2	NS
HR, beats/min	131±4.8*	134±4.4*	133±4.5	+2.4*	NS

*Significantly different from values before propranolol.

and heart rate are likely to be due to blockade of impulses from aortic baroreceptors; blockade of some vagal efferent fibres could have contributed to the increase in heart rate. The effect of aortic baroreceptor impulse discharge on plasma AVP concentration has not been studied, but if they behave like carotid baroreceptors a decrease in discharge would increase plasma AVP concentration (Share 1965). The increase in plasma AVP concentration we observed could have been due to loss of activity from both aortic baroreceptors and atrial receptors. The fact that we completely prevented the decrease in plasma AVP concentration, and the increase in heart rate associated with pulmonary vein distension, by cooling the vagus nerves to 10°C suggests that these reflex responses were mediated through increases in impulse activity in myelinated afferent fibres. Cooling the vagus nerves to 16°C did not cause any significant changes in mean arterial pressure, heart rate, or plasma AVP concentration in our experiments. During distension of the pulmonary vein − left atrial junctions the increase in heart rate was significantly less with the vagus nerves at 16°C, than with the vagus nerves warm, and the decrease in plasma AVP concentration was less, and did not reach statistical significance. These findings are consistent with the observation of Kappagoda *et al.* (1979) that the increase in afferent discharge in myelinated fibres in response to pulmonary vein distension was reduced but not prevented when the vagi were cooled to 16°C.

Although we observed only minor changes in mean arterial pressure during pulmonary vein distension, it is possible that the increase in heart rate could have caused an altered stimulus to the arterial baroreceptors. When the reflex increase in heart rate was prevented by administration of propranolol, pulmonary vein distension still caused a significant decrease in plasma AVP concentration. Administration of propranolol decreased heart rate and caused a small increase in mean left atrial pressure but did not cause a significant change in plasma AVP concentration during the control periods.

Our results are consistent with the view that distension of the pulmonary vein − left atrial junctions causes a decrease in plasma AVP concentration by stimulating left atrial receptors with myelinated afferent fibres. The decrease in plasma AVP concentration is a direct result of stimulation of the receptors and is not secondary to the reflex changes in heart rate caused by pulmonary vein distension.

Acknowledgements

The authors are grateful for the excellent assistance received from Jeannie Sharp and David Morton.

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Pflügers Arch (1984) 400:381–387

Pflügers Archiv
European Journal of Physiology

The relationship between plasma vasopressin concentration and urinary excretion during left atrial distension in anaesthetized dogs

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Abstract. The relationship between the changes in plasma vasopressin (AVP) concentration and urinary concentration during left atrial distension has been examined in 12 anaesthetized dogs. Left atrial pressure was increased by 1.2 kPa for 30 min. Plasma AVP concentration (radioimmunoassay) was decreased 5 min after the start of atrial distension and was increased again 5 min after the end of distension. The average decrease was about 50% from a mean of 6.4 ± 2.4 pg · ml^{-1} (SE). Urine osmolality decreased more slowly reaching its lowest value in the first 10 min after removal of atrial distension. In contrast sodium excretion increased immediately upon atrial distension. Because of the difference in the time course of the changes in plasma AVP and urine osmolality, plasma AVP was compared with the urine osmolality in samples collected 15 min after the plasma samples. At any plasma AVP concentration there was a wide variation in urine osmolality between dogs, but in any one dog there was clear relationship between plasma AVP and urine osmolality. The results support the view that the diuretic response to left atrial distension is due, at least in part to decreases in plasma AVP concentration. They also show that a stimulus arising from increased left atrial pressure influences the relationship between plasma osmolality and plasma AVP concentration.

Key words: ADH – Left atrial distension – Chloralose anaesthetized dogs – Urine osmolality – Plasma osmolality – Volume regulation

Introduction

The experiments of Henry et al. (1956) demonstrated that atrial distension in the dog was associated with a diuresis. The slow onset of the effect, its persistence for several minutes after cessation of the stimulus, and the dilution of the urine during the diuresis, suggested a hormone might be involved (Gauer and Henry 1976). Measurements, using radioimmunoassay, of the concentration of arginine vasopressin (AVP) in the plasma of anaesthetized (de Torrente et al. 1973) and unanaesthetized (Schultz et al. 1982; Kaczmarczyk et al. 1983) dogs have demonstrated that plasma AVP concentration decreases during atrial distension.

Most investigators have measured plasma AVP concentration in only single samples taken before, during and after atrial distension (de Torrente et al. 1976; Zucker et al. 1979) but urinary concentration and volume have been shown to change continuously during atrial distension (Lawrence et al. 1973). This has made difficult the interpretation of the diuretic response to left atrial distension in terms of changes in plasma AVP concentration. This relationship is complicated by the fact that the diuresis is accompanied by a natriuresis and increase in osmolal clearance in unanaesthetized (Reinhardt et al. 1980a) and in some anaesthetized (Gupta et al. 1982) dogs. The increases in osmolal clearance and sodium excretion are independent of changes in plasma vasopressin concentration (Ledsome and Mason 1972; Kaczmarczyk et al. 1983). In addition experiments carried out in anaesthetized dogs have been criticized on the basis that the plasma AVP concentrations during atrial distension were higher than those usually associated with maximal urinary concentrations in unanaesthetized dogs (Kappagoda et al. 1974).

The present study was udertaken to examine the relationship between the time course of the changes in plasma AVP concentration and urinary concentration during left atrial distension. In half of the experiments one kidney was denervated in order to examine possible effects of renal nerves on the time course of the diuretic and natriuretic responses.

Material and methods

1. General preparation and surgical procedures

Twelve mongrel dogs weighing 13 to 28 kg (mean 20.3 kg) were given morphine sulphate, 0.5 mg · kg^{-1}, S.C. One hour later a saphenous vein was catheterized and 10 ml · kg^{-1} of α-chloralose solution (BDH Chemicals, Poole, Dorset, GB; 1 g · 100 ml^{-1} in 0.6 w/v NaCl) was infused within 5 min, to induce anaesthesia. As soon as possible thereafter positive pressure ventilation (Model 614, Harvard Apparatus Co., Cambridge, MA, USA) with 40% O_2 in N_2 was begun at a rate of 14 breaths · min^{-1} and a tidal volume of 12.5 ml · kg^{-1} body wt. When the chest was opened an exhalation valve (Ohio Chemical, Madison, WI, USA) provided an expiratory resistance of 0.3 kPa.

Arterial pH, PCO_2 and pH were measured, using standard electrodes (Model 165/2, Corning Glass Works, Medfield, MA, USA), in blood samples of 3 ml drawn at hourly intervals from the femoral artery. The pH was maintained in the range 7.3–7.4 and arterial PCO_2 between 4.5 and 5.2 kPa by adjusting the tidal volume and giving aliquots of $NaHCO_3$ (1 M) i.v. No adjustments were made

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after the experimental protocol was begun. During the surgical procedures 7.7 ml · kg^{-1} of dextran (dextran 70 in 0.9 NaCl, Pharmacia Ltd. Dorval, Quebec, Canada) was infused. After the surgery was completed anaesthesia was maintained with a constant infusion of chloralose (0.5 g · 100 ml^{-1} in 0.6 NaCl at 2 ml · min^{-1}).

A balloon was inserted into the left atrium and recording systems were established as described previously (Ledsome et al. 1982). In 6 dogs the left kidney was denervated (Ledsome et al. 1961). The thoracotomy was left open throughout the experiment.

Experimental protocol

One hour was allowed for stabilization after completion of surgical procedures. During this time acid-base balance was corrected. A recording was then made of arterial pressure, left atrial pressure and heart rate and collection of urine began. After 5 min a sample of arterial blood (10 ml) was taken into a clean dry syringe. The volume of the first sample was replaced with an equal volume of dextran. 7 ml of the blood removed was transferred to a cold EDTA tube, the remaining 3 ml was mixed with 2 drops of heparin. Both samples were immediately centrifuged at 4° C and the plasma separated. The red blood cells so obtained were resuspended in dextran and the mixture used to replace the volume of subsequent blood samples. Urine samples were collected every 10 min and a recording of cardiovascular variables was made. Blood samples were taken at 10 min intervals at the mid point of the urine collection periods. After 30 min warm saline was injected into the balloon in the left atrium to increase left atrial pressure by about 1 kPa. Inflation of the balloon was maintained for 30 min. After deflation, recording and collection of blood samples was continued for a further 40 min. One such test was performed in each animal.

Analysis of plasma and urine samples

Radioimmunoassay (RIA) for plasma AVP was carried out as described earlier (Ledsome et al. 1982) with the exception that all plasma samples underwent extraction prior to measurements. For this series of experiments the extraction recovery was 87% ± 3. The results are corrected for extraction losses. The anti-AVP serum employed (GP-15) showed no cross reactivity with oxytocin, arginine vasotocin, 4-ser 8-ileu oxytocin, or angiotensin I. The limit of dectection defined as 80% of maximum binding was 0.27 ± 0.02 pg AVP (mean, ± SE); 50% of the maximum binding was at 0.83 ± 0.03 pg AVP (mean, ± SE). Intraassay variability was 4.5%, and interassay variability 7.4%. USP pituitary extract was used as the standard. It was in turn compared with synthetic AVP (Spectrum Laboratories, Los Angeles, CA, USA, lot 208062) to express AVP concentrations on weight basis. Plasma and urine were analysed for sodium, potassium and osmolality as described previously (Ledsome et al. 1982).

Analysis of results

Results are presented in the figures as the mean changes in each 10 min period. For further statistical analysis the measurements were grouped into 3 periods. These periods corresponded to the experimental sequence and consisted of: control I (30 min before atrial distension), experimental (30 min during atrial distension), and control II (40 min following atrial distension). The exceptions to this were the variables of urine osmolality, urine volume and free water clearance. There was a time lag in the response of these variables to atrial distension and the experimental period was taken as the average of the last 20 min during atrial distension and the first 10 min following atrial distension. Control II for these variables was the period 10–40 min after the end of atrial distension. Differences between the control and experimental periods were tested using a paired *t*-test.

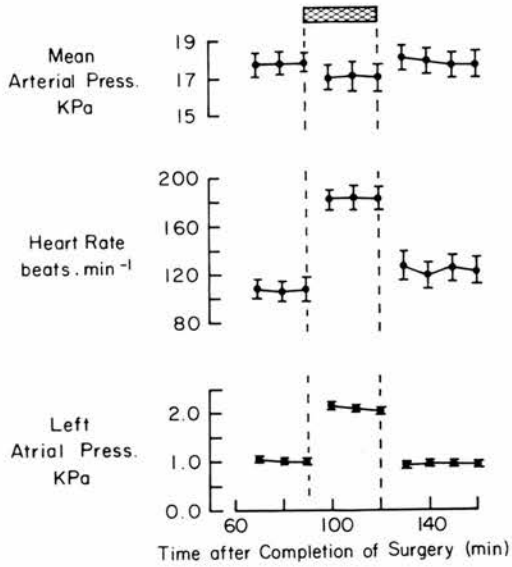

Fig. 1. Left atrial pressure, heart rate and mean arterial pressure before, during and after a period of atrial distension (indicated by the *hatched bar*). Points are mean ± SE (n = 12)

To allow statistical evaluation of the time course of the changes in plasma AVP concentration and urinary excretion, the average values of the first three 10 min periods before atrial distension were compared with the values observed in each of the subsequent 10 min periods using a Student's *t*-test for paired data. Multiple comparison tests were not used as the objective of the experiment was primarily to detect the time of the first apparent rise in the various measures and not to assess the significance of an increase at any point in time. But because seven different variables were being considered and not just one, the probabilities associated with the t values would be underestimated and consequently two consecutive t values at the 5% level were required before the measured increase was considered to be significant.

Results

At the start of the experiments (1 h after completion of the surgical procedures) the plasma osmolality was 291 mosmol · kg^{-1} (± 16.0, SD), plasma sodium concentration was 140 mmol · l^{-1} (± 10.1) and plasma potassium concentration was 3.2 mmol · l^{-1} (± 0.5); there were no significant changes in these variables throughout the experimental period. Inflation of a balloon in the left atrium caused an

increase of 1.26 kPa in mean left atrial pressure. There was a marked increase in heart rate (67 beats · min^{-1}) and a small but significant decrease in mean arterial pressure (0.55 kPa, $P < 0.05$). The time course of the cardiovascular changes, in 12 experiments, is shown in Fig. 1.

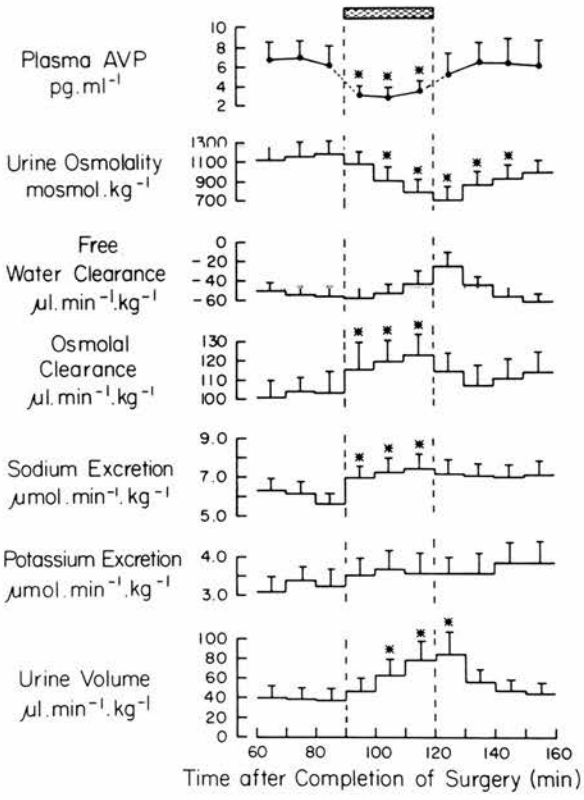

Fig. 2. Changes in plasma AVP concentration and urinary excretion, before, during and after a period of atrial distension (indicated by the *hatched bar*). Values are mean, ± SE ($n = 12$). *Asterisks* indicate values which were significantly different ($P < 0.05$) from the average of the first three values (before atrial distension)

Time course of the changes in plasma AVP and urinary excretion

Plasma AVP concentration reached a lower value within 5 min of atrial distension and then remained relatively unchanged during atrial distension. There was an increase in plasma AVP concentration within 5 min of the removal of atrial distension. The time course of the changes in plasma AVP concentration and urinary excretion is shown in Fig. 2.

Urine volume, urine osmolality and free water clearance did not change in the first 10 min period of atrial distension and maximum changes in these variables occurred in the 10 min period immediately following release of the atrial distension. In contrast sodium excretion and osmolal clearance increased in the first 10 min period of atrial distension. Statistical comparison of values to each 10 min period, during and after atrial distension with the period before atrial distension (Control I) confirmed this pattern (Fig. 2). A statistical comparison of the changes in plasma AVP concentration and urinary excretion during the control and experimental periods is given in Table 1.

The time course of the changes in urinary excretion was unaffected by the denervation of one kidney. A comparison of the pattern of urinary excretion in 6 dogs in which one kidney was denervated is shown in Fig. 3. For clarity, standard errors are not included, but statistical comparison of values for the innervated and denervated kidney did not show any significant differences.

Relationship between plasma AVP concentration and plasma osmolality

No attempt was made to alter plasma osmolality during the experiments and plasma osmolality remained relatively constant in any one dog. There was a wide range of plasma osmolalities (270 – 317 mosm · kg^{-1}). The individual data points for plasma osmolality and plasma AVP concentration during the control and experimental periods are shown in Fig. 4. During the control periods the linear regression line had a slope of 0.28 pg · ml^{-1} · mosm · kg^{-1} and an intercept of 268 mosm · kg^{-1} ($r = 0.54$, $n = 24$). During atrial

Table 1. Plasma AVP concentration and urinary excretion, before during and after left atrial distension. Values (means ± SEM) are given for 12 dogs. d is the mean difference between the average of the control periods and the experimental period. P is the probability. The asterisk (*) indicates the value after atrial distension was significantly different ($P < 0.05$) from the value before atrial distension

	Control I	Experimental	Control II	d	P
Plasma AVP (pg · ml^{-1})	6.7 ± 1.9	3.3 ± 0.9	6.2 ± 2.9	3.1 ± 1.6	< 0.05
Urine volume (µl · min^{-1} · kg^{-1})	39 ± 11.6	75 ± 18.6	50 ± 11.9	31 ± 9.8	< 0.01
Urine osmolality (mosm · kg^{-1})	1,159 ± 139	813 ± 133	934* ± 123	234 ± 50	< 0.001
Free water clearance (µl · min^{-1} · kg^{-1})	−54 ± 8.1	−40 ± 12.3	−54 ± 7.3	13.3 ± 8.6	NS
Osmolal clearance (µl · min^{-1} · kg^{-1})	103 ± 8.9	119 ± 12.7	111 ± 10.7	12.1 ± 6.4	< 0.05
Sodium excretion (µmol · min^{-1} · kg^{-1})	6.1 ± 1.2	7.2 ± 1.5	7.1 ± 1.3	0.6 ± 1.2	NS
Potassium excretion (µmol · min^{-1} · kg^{-1})	3.3 ± 0.4	3.6 ± 0.5	3.8 ± 0.5	0.1 ± 0.5	NS

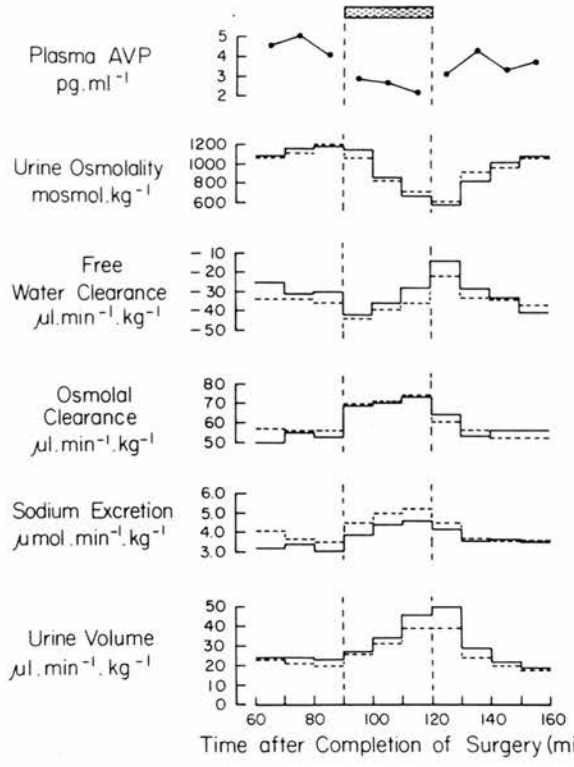

Fig. 3. Changes in plasma AVP concentration and urinary excretion before, during and after a period of atrial distension (indicated by the *hatched bar*). Continuous lines show excretion from the right kidney (*innervated*) and the broken lines show excretion from the left kidney (*denervated*) ($n = 6$)

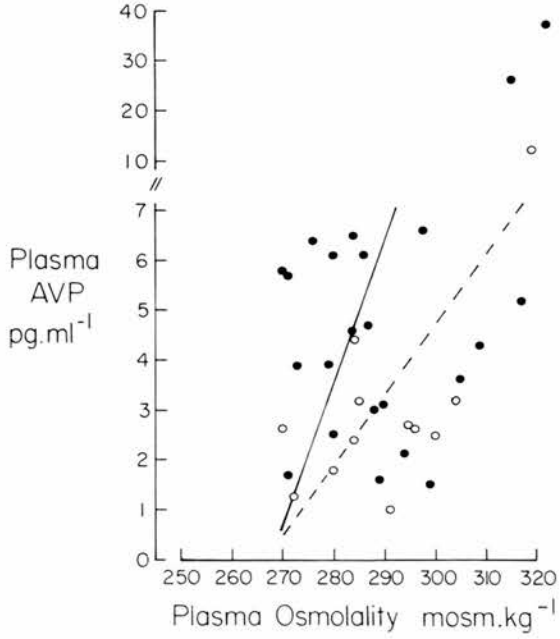

Fig. 4. Plasma AVP concentration and plasma osmolality before, during and after atrial distension. Each of the *closed circles* represents the mean of 3 values in either Control I or Control II. The regression line for these points in the *continuous line* ($n = 24$). The *open circles* are the mean of the 3 values in the experimental period. The *broken line* is the regression line for these points ($n = 12$)

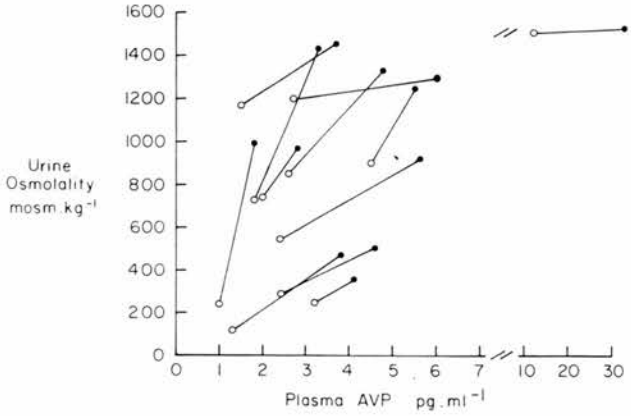

Fig. 5. Plasma AVP concentration and urine osmolality in one test of atrial distension in 12 dogs. The *closed circles* are the means of six control periods (3 periods of Control I and 3 periods of Control II). The *open circles* are the mean of the plasma AVP concentrations during atrial distension and the urine osmolality in the first period following removal of atrial distension. Values from the same experiment are joined

distension plasma AVP concentration was reduced and the regression line had a slope of 0.14 pg · ml^{-1} · mosm · kg^{-1} and an intercept of 266 mosm · kg^{-1} ($r = 0.68$, $n = 12$).

Relationship between plasma AVP concentration and urine osmolality

Because a steady state of urine osmolality was not achieved during atrial distension and because there was a time lag between the changes in plasma AVP concentration and changes in urine osmolality we have compared the average plasma AVP concentration during atrial distension with the urine osmolality in the first period following atrial distension (usually the period of lowest urine osmolality). The data points are plotted in Fig. 5 together with the data points during the control periods. There was a wide variation in the relationship between plasma AVP and urine osmolality between animals, but in every case atrial distension caused a decrease in plasma AVP concentration and urine osmolality. The lowest urine osmolalities (less than 300 mosm · kg^{-1}) were seen only when plasma AVP was less than 3.5 pg · ml^{-1}. Free water clearance became positive during the diuresis in three experiments; in each of these plasma AVP concentration decreased to <2 pg · ml^{-1} during atrial distension. In one animal in which plasma AVP concentration was high (32 pg · ml^{-1}) atrial distension caused a large change in plasma AVP concentration but only a small decrease in urine osmolality.

The relationship between plasma AVP concentration and urine osmolality was more obvious when individual data points were plotted. In Fig. 6 are shown 4 experiments in which plasma AVP concentration decreased to less than 2 pg · ml^{-1}. There was a clear relationship between the plasma AVP concentration and urine osmolality in each experiment.

Discussion

Numerous investigators have confirmed the observation by Henry et al. (1956) that left atrial distension causes a diuresis and natriuresis in anaesthetized dogs (reviewed by Linden

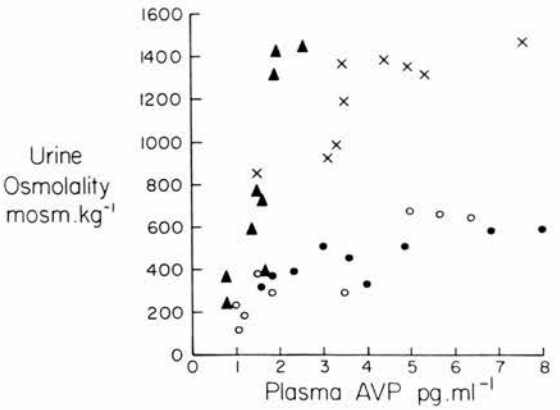

Fig. 6. Individual data points for four experiments, selected to show a range of relationships between plasma AVP concentration and urine osmolality. Each symbol represents points from a single dog. Each point represents the plasma AVP concentration sampled at the mid-point of a collection period plotted against the urine osmolality in the following 10 min collection period

and Kappagoda 1982). A similar diuresis accompanied by a more significant natriuresis occurs in unanaesthetized dogs (Kaczmarczyk et al. 1981, 1983). Measurements of plasma AVP concentrations made during each 10 min urinary collection period have not previously been reported. Frequent measurements of plasma AVP concentrations ensure detection of rapid increases or decreases in plasma AVP (Ledsome et al. 1983). Kaczmarczyk et al. (1983) were unable to show any relationship between plasma AVP concentration and plasma osmolality, urine volume or urine osmolality in unanaesthetized dogs in which plasma samples were taken at hourly intervals.

The responses to atrial distension reported here were similar to those which have been described previously in anaesthetized dogs (e.g. Ledsome and Mason 1972). The rapid decrease in plasma AVP concentration at the start of atrial distension was consistent with the changes observed when sampling was made at 2 min intervals (Ledsome et al. 1983). The time course of the changes was consistent with the data of Weitzman and Fisher (1978) showing a rapid component of the plasma clearance of AVP in conscious dogs and with the changes shown by Wade et al. (1982) in response to an osmotic stimulus. Despite the rapid change in plasma AVP concentration' urine volume and osmolality did not change significantly until 10–20 min after the start of atrial distension. Maximum changes in urine volume and osmolality occurred in the first period after atrial distension; plasma AVP concentration had increased to close to the predistension level at the mid point of this period. The differences in the time courses of the changes in plasma AVP concentration and urine volume and osmolality make difficult the correlation of the plasma AVP with urine volume and osmolality.

Atrial distension was accompanied by a natriuresis which began in the first 10 min period of atrial distension. After removal of atrial distension sodium excretion did not return to predistension levels. An elevation of sodium excretion after the end of atrial distension has previously been noted in some conscious dogs (Reinhardt et al. 1980b). The increases in sodium excretion and osmolal clearance have been shown to be unaffected by infusion of vasopressin, whereas increases in free water clearance (seen in 5 of 12 tests in the present series) were prevented by vasopressin infusion (Ledsome and Mason 1972; Kaczmarczyk et al. 1983). The time courses of the changes in urine osmolality, free water clearance, osmolal clearance and sodium excretion were unaffected by denervation of one kidney (Fig. 3). Thus, although left atrial distension is known to cause a decrease in renal vascular resistance (Mason and Ledsome 1972) and a decrease in renal nerve sympathetic activity (Karim et al. 1972) the renal nerves do not appear to be necessary either for the dilution of the urine or the natriuresis which accompanies left atrial distension in the anaesthetized dog. The mechanism for the natriuresis remains unknown (Kaczmarczyk et al. 1983).

In the majority of previous investigations in anaesthetized dogs (Linden and Kappagoda 1982) plasma AVP concentrations were found to be higher than 10 pg $\cdot$ ml^{-1} during atrial distension. This has led to the suggestion that the relationship between plasma AVP concentration and urine osmolality may be different in the anaesthetized, as compared to the unanaesthetized dog (Kappagoda et al. 1974). The urinary concentrating ability of AVP is altered by the degree of hydration (Perlmutt 1961, 1962) so that even after continual infusion of vasopressin for 150 min urine concentration may be less in severely hydrated than in mildly hydrated dogs. Such studies imply that differences may be expected to occur between individual dogs in their plasma AVP/urine osmolality (pAVP/U_{osm}) relationship, reflecting the state of the animal's hydration. In the present experiments (Figs. 5, 6) there was a wide range of urine osmolalities associated with any plasma AVP concentration. The lowest urine osmolalities after atrial distension were associated with the lowest values of plasma AVP. In any one experiment there was a clear relationship between plasma AVP concentration and urine osmolality (Fig. 6) and the largest changes in urine osmolality occurred in the range of plasma AVP of 1–6 pg $\cdot$ ml^{-1} which has been associated with changes in urinary concentration in normal, hydrated, unanaesthetized dogs (Bie 1980).

It is not clear why the plasma AVP concentrations in this series of experiments were less than those found by previous authors in anaesthetized dogs. Chloralose anaesthesia, by itself does not lead to an increase in plasma AVP concentration (Ginsberg and Brown 1956) and surgical trauma, not associated with blood loss, does not necessarily cause an increase in plasma AVP concentration in human subjects (Crone et al. 1982). The latter series was carried out using the same assay as in the present experiments. Similar results, indicating that neither anaesthesia nor surgical stimulation stimulate AVP release in humans have been reported by other laboratories (Stanley et al. 1979; Philbin et al. 1979; Kono et al. 1981). The effects of anaesthesia on AVP in humans have been reviewed (Oyama 1980; Philbin and Coggins 1980). Similar results have recently been described in dogs anaesthetized with pentobarbitone (Abed et al. 1982); in these experiments plasma AVP was increased only when there was hypotension secondary to haemorrhage or when there was hypoxia. We were careful to minimize blood loss during surgery and to replace volume lost by sampling. Administration of 40% O_2 ensured PaO_2 was always >100 mm Hg. We have completed two other series in which the plasma AVP concentrations were within the same range as the present (Wilson and Ledsome 1983; Ledsome et al. 1983). If there is an abnormally high release of AVP in the

chloralose anaesthetized dog it might be expected that the relationship between plasma AVP and plasma osmolality would be abnormal. Although the correlation between plasma AVP and plasma osmolality was poor, the regression line during the control periods, had a slope similar to that described by Quillen and Cowley (1983) in conscious normovolaemic dogs (0.28 pg · ml^{-1} · mosm · kg^{-1} in our experiments, as compared to 0.21 of Quillen and Cowley 1983). The slope decreased with atrial distension to a value similar to that seen by Quillen and Cowley (1983) when atrial pressure was increased by 0.6 kPa with hypervolaemia (0.14 pg · ml^{-1} · mosm · kg^{-1} in our experiments, as compared to 0.16). The intercepts in our anaesthetized dogs (268 mosm and 266 mosm), were slightly lower than those (270 mosm in hypovolaemia and 281 mosm in hypervolaemia) obtained by Quillen and Cowley (1983). The results support the view that there is no difference in the relationship between plasma osmolality and plasma AVP concentration in the chloralose anaesthetized as compared to the unanaesthetized dog. Our results showing that left atrial distension causes changes in the relationship of pAVP/P_{osm} similar to those of hypervolaemia, support the conclusion of Quillen and Cowley (1983) that left atrial pressure changes are a major determinant of these responses to hypervolaemia.

It is concluded that left atrial distension causes a rapid and sustained decrease in plasma AVP concentration. In the hydrated, chloralose anaesthetized dog, plasma AVP concentration may be within the range seen in unanaesthetized dogs (Bie 1980) and the changes observed in urine osmolality with atrial distension are appropriate for the observed changes in plasma AVP concentration. The results support the view that a stimulus arising from increased left atrial pressure influences the relationship between plasma osmolality and plasma AVP concentration. They are consistent with the hypothesis that the diuretic response which accompanies left atrial distension in anaesthetized dogs, is due, at least in part to decreases in plasma AVP concentration.

Acknowledgements. We thank Jeannie Sharp and David Morton for their technical assistance, and Dr. Brenda Morrison for advice regarding statistics. The work was supported by grants from the MRC of Canada and the B. C. Heart Foundation.

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Received July 26/Accepted December 23, 1983

Plasma vasopressin during increases and decreases in blood volume in anaesthetized dogs[1]

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Received July 9, 1984

LEDSOME, J. R., N. WILSON, and C. A. COURNEYA. 1985. Plasma vasopressin during increases and decreases in blood volume in anaesthetized dogs. Can. J. Physiol. Pharmacol. **63:** 224–229.

In chloralose anaesthetized dogs, plasma vasopressin concentration was measured by radioimmunoassay during step changes in blood volume of 4 mL/kg over a range of blood volume from +20 to −12 mL/kg. Blood volume was both increased and decreased over this range. There was a logarithmic relationship between blood volume and plasma vasopressin concentration over the range of blood volume examined. There was also a logarithmic relationship between blood volume and mean left atrial pressure. Linear regression between the natural logarithm of plasma vasopressin concentration and mean arterial pressure, heart rate, and mean left atrial pressure gave the highest correlation coefficient ($r = 0.94$) between vasopressin and mean arterial pressure. The results support the hypothesis that there are sensitive mechanisms controlling the release of vasopressin in response to changes in blood volume. Observations were also made of changes in atrial pressure and activity of left atrial receptors during changes in blood volume over the same range. The results suggest that changes in atrial receptor activity are unlikely to be the major cause of the large increases in plasma vasopressin concentration associated with hypovolemia.

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On a déterminé par radio immunodosage, chez des chiens anesthésiés au chloralose, la concentration de vasopressine plasmatique lors de variations en échelon de volume sanguin de 4 mL/kg, sur une plage de volume sanguin de +20 à −12 mL/kg. On augmenta et on diminua le volume sanguin sur cette plage. Il y avait une relation logarithmique entre les concentrations de volume sanguin et de vasopressine plasmatique sur la plage de volume sanguin examiné. Il y avait aussi une relation logarithmique entre le volume sanguin et la pression auriculaire gauche moyenne. On établit des régressions linéaires entre le logarithme naturel de la concentration de vasopressine plasmatique et de la pression artérielle moyenne, de la fréquence cardiaque et de la pression auriculaire gauche moyenne; on obtint le plus haut coefficient de corrélation ($r = 0.94$) entre la vasopressine et la pression artérielle moyenne. Les résultats supportent l'hypothèse de l'existence de mécanismes sensibles contrôlant la libération de vasopressine en réponse aux variations de volume sanguin. On observa en outre les variations de pression auriculaire et d'activité des récepteurs auriculaires gauches pendant les variations de volume sanguin sur cette même plage. Les résultats suggèrent que les variations d'activité des récepteurs auriculaires ne sont vraisemblablement pas la cause principale des fortes augmentations de concentration de vasopressine plasmatique associées à l'hypovolémie.

[Traduit par le journal]

Introduction

Haemorrhage is associated with marked increases in plasma vasopressin (AVP) concentration in anaesthetized (Clark and Rocha E Silva 1967; Claybaugh and Share 1973) and unanaesthetized animals (Wang et al. 1983). There are fewer reports of the effects of increasing blood volume, but it is clear that an increase in blood volume is associated with a decrease in plasma AVP concentration (Zehr et al. 1969; Shade and Share 1975; Wade et al. 1983). These observations led to the hypothesis that there is control of plasma AVP concentration sensitive to small changes in blood volume over a physiological range (Shade and Share 1975). The hypothesis has been criticized because in many experiments plasma AVP concentration in the control state has been high (Share 1968) and outside the normal range of 1–10 pg/mL (Bie 1980); there has been variability in the response in different animals (Henry et al. 1968); volumes of blood removed during haemorrhage have been large (Share 1968; Henry et al. 1968); and infusions have been of isosmotic electrolyte solutions rather than blood (Zehr et al. 1969; Shade and Share 1975), so that the actual change in blood volume or extracellular fluid volume has been difficult to assess.

The aim of the present experiments was to examine, in anaesthetized dogs prepared so that the plasma AVP concentration was within the range of 1–10 pg/mL, the relationship between plasma AVP concentration and alterations in blood volume. The responses to increasing and decreasing blood volume were examined. Decreases in blood volume were limited to 12 mL/kg to avoid haemorrhagic shock. In a similar preparation, impulses were recorded from single functional fibres in the cervical vagus nerves, arising from left atrial receptors, whilst blood volume was changed over the same range. Thus it was possible to estimate the changes in atrial receptor activity which may have accompanied the observed changes in atrial pressure during infusion and haemorrhage.

Methods

Twenty-one mongrel dogs of 15–30 kg were given morphine sulphate (0.5 mg/kg, sc). One hour later a saphenous vein was catheterized and 10 mL/kg of α-chloralose solution was infused, within 5 min, to induce anaesthesia (BDH Chemicals, U.K.; 1 g/100 mL in 0.9% (w/v) NaCl). As soon as possible thereafter positive pressure ventilation (model 614, Harvard Apparatus Co., MA) with 40% O_2 in N_2 was begun at a rate of 14 breaths/min and a tidal volume of 50 mL/4 kg body weight. When the chest was opened, an expiratory resistance of 3 cmH_2O was provided.

Arterial pH, P_{CO_2}, and P_{O_2} were measured with appropriate electrodes (model 165/2, Corning Glass Works, Medfield, MA) in blood samples of 3 mL drawn at approximately hourly intervals from the femoral artery. The pH was maintained in the range 7.3–7.4 and arterial P_{CO_2} between 35 and 40 mmHg (1 mmHg = 0.13kPa) by adjusting the tidal volume and by giving aliquots of $NaHCO_3$ (1 *M*) iv. No adjustments were made after the protocol was begun. During the surgical procedures each animal received 7.7 mL/kg of dextran (Dextran 70 in 0.9% NaCl, Pharmacia Ltd., Québec) and 20 mL/kg

[1]Supported by the Medical Research Council of Canada, the British Columbia Heart Foundation and the B.C. Health Care Research Foundation.

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of NaCl (0.5 g/100 mL) was infused at a rate of 7 mL/min. After surgery was completed anaesthesia was maintained with a constant infusion of chloralose (0.5 g/100 mL saline at 2 mL/min). The chest was opened in the left fifth intercostal space and left atrial pressure was recorded through a stainless steel cannula (2 mm bore) placed in the middle left pulmonary vein. That portion of the left lung draining blood through this pulmonary vein was tied close to the lung root. In some experiments a balloon was placed in the left atrium through the appendage (Ledsome et al. 1961) to allow rapid manipulation of atrial pressure for identification of atrial receptors. Femoral arterial pressure was recorded from a 15-cm length of Teflon tubing of 1-mm bore. Both cannulas were connected to strain gauge transducers (P23dB, Statham Inst Co., Puerto Rico). Calibration was performed in stepwise fashion using water and mercury manometers. Zero left atrial pressure was referred to the cannula tip, free in air, at the end of the experiment. Mean pressures were obtained using a resistance–capacitance circuit with a time constant of 2 s. Heart rate was counted from an electrocardiogram over intervals of 30 s.

In 11 experiments the sheath was removed from about 5 cm of the left vagus nerve in the neck. Small slips of the nerve were cut proximally and placed on bipolar platinum electrodes, the signal was amplified (model P15, Grass Inst. Co., Millis, MA) and displayed on an oscilloscope (model 565, Tektronik, Portland, OR). The nerve was dissected until single functional fibres were obtained which demonstrated activity characteristic of type-B (Paintal 1953) left atrial receptors. In some cases this involved dissection of a small part of the vagus nerve and in other experiments only one or two slips were cut. The signal was analysed using a window discriminator (N-750, Mentor Corporation, Minneapolis, MI) and impulses were counted using a digital counter (5300, Hewlett-Packard, Palo Alto, CA). At the end of the experiment the atrium was opened widely and the endocardium probed with a fine glass rod. Fibres were accepted as arising from left atrial receptors only if a high frequency discharge was obtained by gently probing a limited area of the endocardium with a fine glass rod (Kidd et al. 1978).

Experimental protocol

Immediately after the surgical procedures had been completed the animal was heparinized (Heparin sodium, 500 IU, Nutritional Biochemicals, Cleveland, OH). Dextran was delivered by means of a roller pump through a 4-mm bore stainless steel cannula, into the right femoral artery, at a rate of 240 mL/min until a volume of 20 mL/kg dextran had been infused. After 1 min the same volume was removed by reversing the pump and pumping at the same rate. After another minute this process of infusion and withdrawal was repeated. This procedure provided a volume of 20 mL/kg of a blood and dextran mixture which allowed infusion or withdrawal from the animal without alteration in the composition of the circulating blood. After the exchange was complete a period of 60 min was allowed before any measurements were made. During this time the blood–dextran mixture was kept in a water bath at 37°C.

In eight experiments in which the vagus nerves were intact, measurements were made of left atrial pressure, femoral arterial pressure, and heart rate. A sample of 10 mL of blood was taken from the femoral artery, 7 mL was transferred into an EDTA tube (6450, Becton Dickinson, Canada) for measurement of plasma AVP concentration, the other 3 mL was mixed with two drops of heparin for measurement of plasma osmolality, sodium concentration, potassium concentration, and haematocrit. The blood–dextran mixture was then pumped into the femoral artery at a rate of 240 mL/min until 20 mL/kg body weight had been infused. A record of cardiovascular variables was immediately taken. Five minutes later another record was taken and a second blood sample was removed. Blood was then removed to decrease blood volume by 4 mL/kg and the measurements were repeated. The process was repeated so that measurements were made, in sequence, with normal blood volume, blood volume increased by 20, 16, 12, 8, and 4 mL/kg; normal blood volume, blood volume decreased by 4, 8, 12, 8, and 4 mL/kg; normal blood volume and blood volume increased by 4, 8, 12, 16, and 20 mL/kg. The sequence of these 18 measurements is shown in Fig. 1. The volume of the first

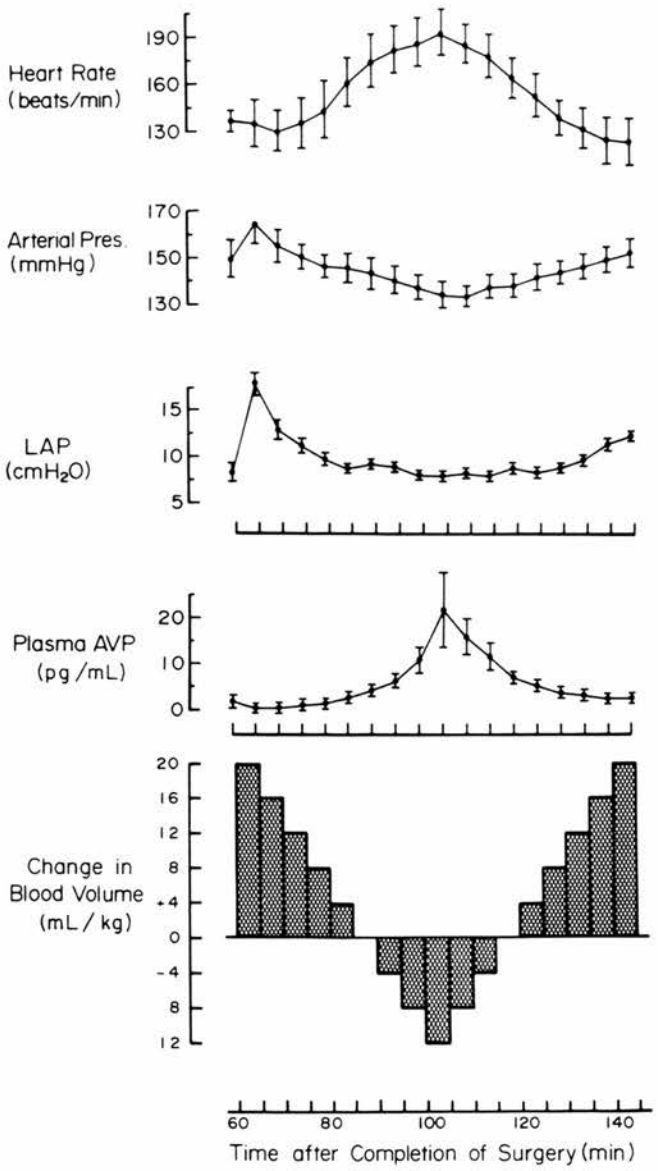

FIG. 1. Diagram illustrating the protocol of the experiment. Blood volume was altered every 5 min. Heart rate, mean arterial pressure, left atrial pressure (LAP), and plasma AVP concentration were measured 5 min after each change in blood volume. (n = 6.)

blood sample was immediately replaced with dextran. Each blood sample was immediately centrifuged at 4°C and the red cells were resuspended in dextran. Subsequent blood samples were replaced with an equal volume of the dextran – red cell mixture. In two additional experiments the blood–dextran exchange was made and blood samples were taken at the same intervals as described above, but no changes in blood volume were made. These experiments were time controls for changes in plasma AVP concentration.

In another 11 experiments the surgical preparation was similar but activity was recorded from a single functional fibre in the left vagus nerve arising from a receptor in the left atrium. Blood volume was increased and decreased in steps of 4 mL/kg over the same range of blood volume as in the previous experiment. Atrial receptor activity was recorded 4 min after each change in blood volume. Rapid adaptation of atrial receptors to a change in atrial pressure was complete at this time (Ledsome and McFetridge 1984). Plasma AVP was not measured in these experiments because interruption of vagal afferents may have influenced the plasma AVP.

Analysis of blood samples

The radioimmunoassay (RIA) for plasma AVP was carried out as

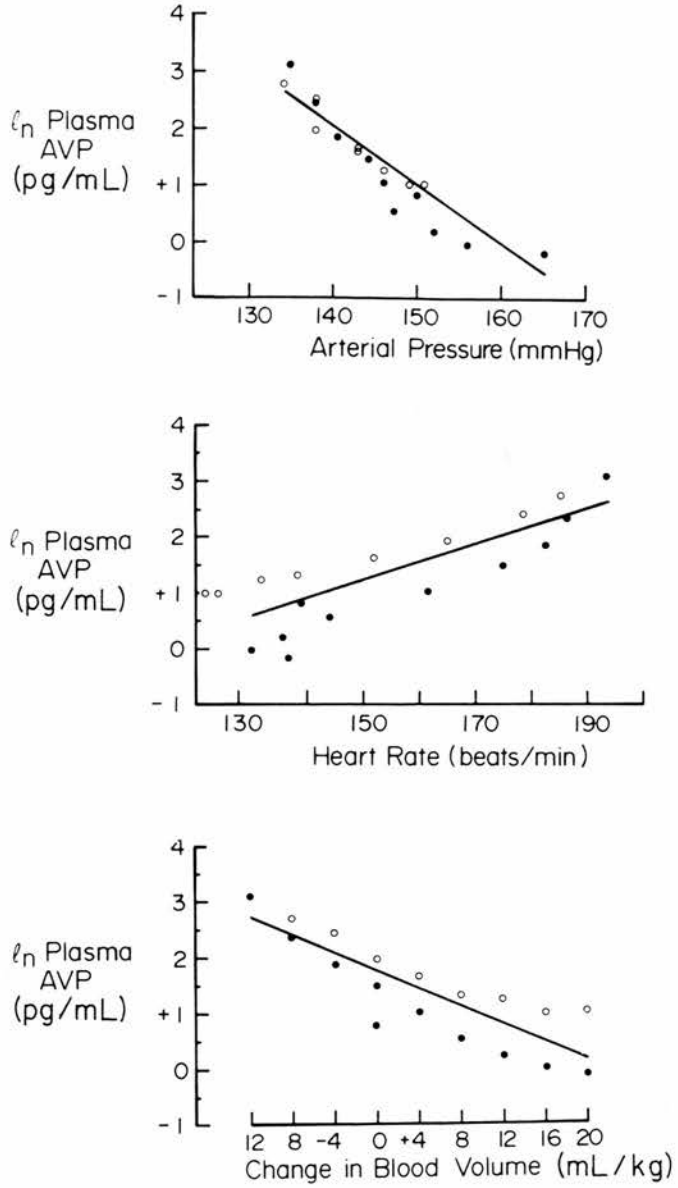

FIG. 2. The relationship between the natural logarithm of plasma AVP concentration and mean arterial pressure, heart rate, and change in blood volume. Closed circles include the first measurement and measurements made during decreases in blood volume from +20 mL/kg. Open circles are measurements made during increases in blood volume from −12 to +20 mL/kg.

described earlier (Ledsome et al. 1982). For this series of experiments the extraction recovery was 81.9 ± 8.7% (n = 10, mean ±SD). The results presented have been corrected for extraction losses. The limit of detection, defined as 80% of maximum binding was 0.25 ±0.05 pg AVP. Fifty percent of maximum binding was at 0.82 ± 0.02 pg AVP. In terms of plasma AVP concentrations, the average minimal detectable level was 0.5−0.7 pg/mL. This was achieved by using 350 μL of plasma extract per assay tube (total incubation volume 1.0 mL) and by ensuring that the final volume of the plasma extract was less than the volume of the plasma specimen used for extraction. The average extract volume remaining after the evaporation of organic solvents was always 5−20% smaller than the starting volume of plasma.

Plasma was analysed for sodium, potassium, and osmolality as described previously (Ledsome et al. 1982). Haematocrit was measured using a microhaematocrit centrifuge.

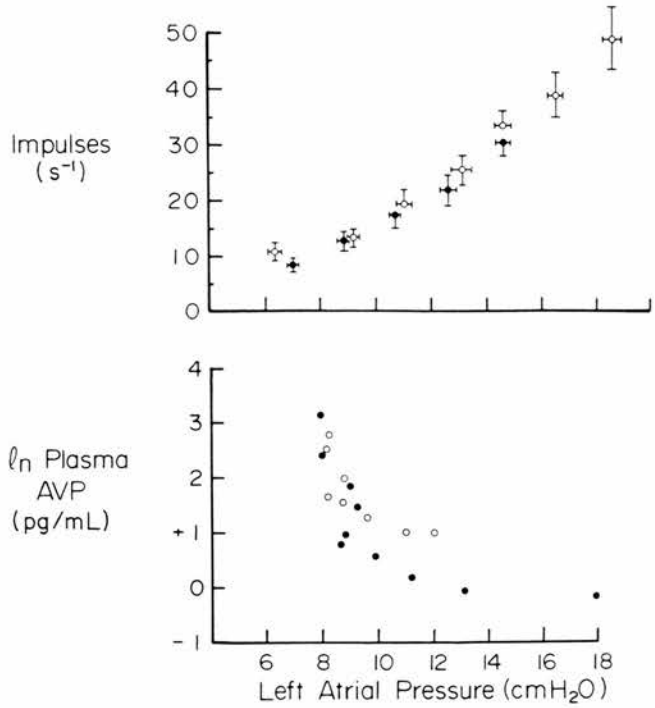

FIG. 3. Changes in mean left atrial pressure during increases and decreases in blood volume compared to the natural logarithm of plasma AVP concentration measured in 6 dogs and the rate of impulse discharge from left atrial receptors, measured in 11 other dogs. Bars in the upper panel are SE of the mean. Other conventions as in Fig. 1.

Results

At the start of the experiments plasma osmolality was 293 ± 9.9 mosmol/kg (mean ± SD), plasma sodium concentration was 148 ± 10 mequiv./L, and plasma potassium concentration was 3.4 ± 0.3 mequiv./L; the haematocrit was 28.5% ± 6.2%. Five minutes following the rapid infusion of 20 mL/kg of the blood−dextran mixture there was no change in any of these four variables. During the 90-min experimental period there was a gradual decrease in osmolality (to 284 ± 12 mosmol/kg), a gradual increase in sodium concentration (to 154 ± 14 mequiv./L), and a gradual increase in potassium concentration (to 3.7 ± 2.7 mequiv./L). The changes between the values of these variables at the start and the end of the experiment were not statistically significant and there were no consistent changes which were related to the volume infused or withdrawn. Haematocrit increased gradually to reach 36 ± 6.4% at the time blood volume was lowest (−12 mL/kg) and decreased again during the reinfusion to reach 26 ± 5.2% at the end of the experiment. The difference between the haematocrit at the start of the experiment and at the time when the blood volume was lowest was statistically significant ($p < 0.05$). No attempt was made to calculate and correct blood volume for the apparent change in plasma volume indicated by the changes in haematocrit.

The changes in left atrial pressure, arterial pressure, heart rate, and plasma AVP concentration during infusion and withdrawal of blood in six dogs are shown in Fig. 1. Plasma AVP concentration at the start of the experiment was 2.3 ± 0.6 (SEM) pg/mL. Increasing blood volume by 20 mL/kg caused an increase in atrial pressure and femoral arterial pressure and

a decrease in plasma AVP to 0.8 ± 0.3 pg/mL; this difference was significant ($p < 0.001$). Blood volume was then reduced in steps; atrial pressure and arterial pressure decreased with decreasing blood volume and increased with increasing blood volume. As blood volume was decreased from +20 mL/kg, plasma AVP concentration increased; between +20 and +4 mL/kg a change of 8 mL/kg was needed to produce a significant change in plasma AVP concentration ($p < 0.05$). When blood volume was less than +4 mL/kg a change in blood volume of 4 mL/kg was sufficient to cause a significant change in plasma AVP concentration. Reinfusion reversed the effects of decreasing blood volume, but because the maximum blood volume of 20 mL/kg above the starting blood volume was approached more slowly than with the first infusion, atrial pressure increased to 12.1 cmH_2O compared with 17.8 cmH_2O with the first infusion. Arterial pressure rose to 151 mmHg compared with 165 mmHg. Plasma AVP concentration decreased to 2.8 pg/mL compared with 0.8 pg/mL. Heart rate at the start of the experiments was 139 beats/min. Following the initial infusion of 20 mL/kg of the blood−dextran mixture, heart rate increased in three dogs and decreased in three dogs. Removal of blood in steps from +16 to −12 mL/kg caused a gradual increase in heart rate to reach a maximum heart rate of 194 beats/min. Subsequent reinfusion caused a decrease in heart rate.

Because the changes in plasma AVP concentration may have been influenced by the cardiovascular changes immediately after each volume change, rather than by the values present at the time of sampling 5 min later, cardiovascular variables were recorded immediately after the volume change. Left atrial pressure and arterial pressure were higher (25 cmH_2O and 179 mmHg, respectively) immediately after the first infusion of 20 mL/kg than they were 5 min later. With the step changes of 4 mL/kg there were only small and not significant differences between the values immediately after the volume change and those observed 5 min later. We have therefore used the values measured at the time of sampling, 5 min after the volume change in our analysis.

Further analysis of the results (Fig. 2) showed that there was a linear correlation between the natural logarithm of the plasma AVP concentration and blood volume ($r = 0.86$, $p < 0.001$), heart rate ($r = 0.82$, $p < 0.001$), and mean arterial pressure ($r = 0.94$, $p < 0.001$). The relationship between the natural logarithm of the plasma AVP concentration and left atrial pressure (Fig. 3) yielded a lower linear correlation coefficient ($r = 0.75$), although this was still statistically significant ($p < 0.001$). The relationship between blood volume and left atrial pressure was logarithmic (Fig. 1). Linear regression between blood volume and the natural logarithm of mean left atrial pressure gave a regression coefficient (r) of 0.86. The apparent hysteresis in the relationship between plasma AVP and the change in blood volume may have been due to a gradual increase in plasma AVP with time (see below) or due to a steady state not being reached within 5 min of the expansion to 20 mL/kg. The slopes of the regression lines during infusion were not statistically different from those during withdrawal.

Results from two of the eight experiments were not included in this analysis. In one experiment the plasma AVP values at the start of the experiment were an order of magnitude greater than in the six experiments reported (18.5 pg/mL at the start of the experiment, decreased to 13.7 pg/mL on volume expansion and increased to 221 pg/mL on haemorrhage to −12 mL/kg). These plasma AVP concentrations lay outside the "normal" range as defined in our objectives. There were no differences in plasma osmolality, sodium concentration, potassium concentration, haematocrit, atrial pressure, arterial pressure, or heart rate which gave any indication of the reason for the increased plasma AVP concentration in this experiment. In another experiment decreasing blood volume from −8 to −12 mL/kg caused haemorrhagic shock indicated by a fall in arterial pressure to 50 mmHg. Until that step the animal had not appeared different from the others. Plasma AVP concentration at the time of the low arterial pressure was greater than 500 pg/mL.

In two additional experiments which acted as time controls, dextran and blood were exchanged, but no changes in blood volume were made. Mean arterial pressure and mean left atrial pressure were unchanged during the 90 min of the protocol. Heart rate increased gradually from 156 to 212 beats/min and haematocrit increased from 29 to 31%. Plasma AVP concentration was 1.8 pg/mL at the start of the experiments and gradually increased to 3.7 pg/mL 90 min later.

Measurements were obtained in 11 animals of atrial receptor activity and left atrial pressure during changes in blood volume similar to those described above. The relationship between mean left atrial pressure and impulse activity (impulses per second) is shown in Fig. 3. The points plotted were obtained by grouping observations into steps of atrial pressure of 2 cmH_2O and averaging the atrial pressure and impulse activity in the observations within each step. Each point represents the mean of between 7 and 13 observations. In the lower panel of Fig. 3 these observations may be compared to the relationship between left atrial pressure and the natural logarithm of the plasma AVP concentration. A regression line has not been drawn on this plot because of the relatively nonlinear nature of the relationship. It should be noted that the observations on impulse discharge were made in different animals from those in which plasma AVP was measured.

Discussion

At the start of the experimental period the plasma AVP concentration was 2.3 pg/mL and was within the range of 1−10 pg/mL found in normally hydrated conscious dogs (Bie 1980). This was consistent with the plasma osmolality of 294 mosmol/kg which was achieved by the hydration procedure. Wietzman and Fisher (1977) using sheep and Wade et al. (1982) using dogs have shown that at plasma osmolalities below 300 mosmol/kg plasma vasopressin is usually below 2 pg/mL and there is little further decrease with decreasing osmolality. In the present experiments volume expansion of 20 mL/kg produced a further significant decrease in plasma AVP concentration. The infusion of this volume of a blood−dextran mixture produced no changes in haematocrit, plasma osmolality, sodium or potassium concentration and it is therefore likely that the prior mixing of the blood and dextran had been complete and that the decrease in plasma AVP was due to the change in volume and not the composition of the plasma. When blood volume was changed in small steps (4 mL/kg) the changes in plasma AVP from one step to the next were significantly different between +20 and +4 mL/kg when an 8 mL/kg change in blood volume was achieved. At blood volumes below +4 mL/kg a change of 4 mL/kg was sufficient to produce statistically significant changes in plasma AVP. The linear correlation between the change in blood volume and the

logarithm of plasma AVP (Fig. 2) is strong evidence that plasma AVP was responding to the changes in blood volume over the whole range of blood volume tested. It was not possible to detect a "threshold" volume below which decreases in blood volume increased plasma AVP and above which there was no effect. The relationship between blood volume and plasma AVP concentration, over the range tested, was best expressed as a logarithmic function, in agreement with the findings of Shade and Share (1975). The results confirm the opinion of Claybaugh and Share (1973) and Shade and Share (1975) that the mechanisms controlling the release of vasopressin in response to blood volume are extremely sensitive.

Stimulation of either atrial receptors (Wilson and Ledsome 1983) or arterial baroreceptors (Thames and Schmid 1981) has been shown to influence plasma AVP concentration, but the relative influence of the two sets of receptors is uncertain. In the dog changes in left atrial pressure have been thought to be of more importance than changes in arterial pressure in influencing plasma AVP during haemorrhage (Henry et al. 1968; Claybaugh and Share 1973). These conclusions have been based on the observation that these authors found no change in mean arterial pressure during haemorrhage. The changes in atrial pressure observed by these authors for a 10% (8 mL/kg) decrease in blood volume were only slightly greater than the 1.3 cmH_2O difference observed in the present experiments over the same range of volume. Although a decrease in atrial pressure of 2 cmH_2O may be associated with a 50% decrease in atrial receptor activity (e.g., from 14 to 7 impulses/s, Fig. 3) (Henry et al. 1968) and further decreases in atrial pressure may result in further decreases in impulse activity, the change is overemphasized by calculating percentage changes and should be considered with reference to the fact that atrial receptor activity can increase up to about 50 impulses/s with increasing atrial pressure. The present results (Fig. 3) indicate that the greatest changes in plasma AVP concentration occurred over a relatively small range of atrial pressures which were likely to be associated with small changes in atrial receptor activity. These data suggest that decreased input from atrial receptors may not be the primary cause of the large increases in plasma AVP concentration seen in hypovolemia. The decreases in plasma AVP concentration which occur when volume is expanded above normal could be dependent upon increased discharge from both atrial receptors and arterial baroreceptors.

There were no data available which allowed us to compare mean left atrial pressure with the expected discharge from left atrial receptors in the dog. However, the data in Fig. 3, which show an increase of about 8 impulses/s for an increase of 2 cmH_2O in mean left atrial pressure, are comparable to the results of Zucker and Gilmore (1976) who plotted left atrial peak "v" wave pressure. There are some difficulties in comparing absolute left atrial pressure reported by different authors in different preparations. Even with the chest open, as in the present experiments, choice of the zero reference level influences the absolute value of the atrial pressure. The present experiments and those of Zucker and Gilmore (1976) employed a cannula inserted into a pulmonary vein and the zero at the cannula tip was close to the posterior wall of the left atrium. The pressures registered are 4–5 cmH_2O higher than when the cannula tip (zero) is in the atrial appendage. It was important to record atrial pressure and atrial receptor activity in a similar preparation to that in which the volume changes were assessed.

In the present experiments the degree of correlation between the mean arterial pressure and the natural logarithm of plasma AVP concentration was greater than that between atrial pressure and the natural logarithm of plasma AVP concentration. Most of the changes in arterial pressure occurred over a range of less than 15 mmHg, but were accompanied by large changes in heart rate suggesting that there was a considerable change in arterial baroreceptor input. Thus changes in arterial baroreceptor input could have contributed to the changes in plasma AVP concentration. Further experiments are required to clarify the relationship between blood volume, stimulation of atrial receptors, aortic baroreceptors, and carotid arterial baroreceptors and plasma AVP concentration.

It has been suggested that other species may differ from dogs in that liberation of vasopressin during haemorrhage requires a fall in arterial pressure. In humans Goetz et al. (1974) showed that a haemorrhage of 10% of the blood volume was not associated with a fall in arterial pressure and there was no increase in plasma vasopressin concentration. More recently Arnauld et al. (1977) showed an exponential relationship between the change in plasma vasopressin concentration and the change in mean arterial pressure following haemorrhage in the conscious monkey. Differences reported between dogs and other species in their response to haemorrhage may depend upon experimental conditions more than upon a true species difference.

Whatever the mechanisms for the release of AVP during volume change, it appears that changes in AVP concentration do occur over a range of at least +20 to −12 mL/kg of blood volume. The relationship between blood volume and plasma AVP is a logarithmic one, providing decreases in plasma AVP concentration in response to increasing blood volume and larger increases in plasma AVP concentration in response to decreasing blood volume. These changes can occur in anaesthetized dogs with normal values of plasma AVP concentration. The effects of a decrease in plasma AVP concentration from this normal state are likely to be mainly upon the renal concentrating mechanism, whereas the larger increases in plasma AVP concentration which accompany decreasing blood volume may have effects upon vascular resistance (Cowley 1982).

Acknowledgements

The authors are grateful for assistance from Jeannie Sharp and Anna-Maria Azzarolo. The work was supported by grants from the Medical Research Council of Canada and the British Columbia Heart Foundation.

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Life Sciences, Vol. 36, pp. 1315-1330 Pergamon Press
Printed in the U.S.A.

MINIREVIEW

ATRIAL RECEPTORS, VASOPRESSIN AND BLOOD VOLUME IN THE DOG

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Summary

Recent work has clarified the relationship between stimulation of left atrial receptors and plasma vasopressin concentration (pAVP) and has allowed a rational explanation of a number of previously anomalous findings. There is now good evidence that mitral obstruction causes a decrease in pAVP and that the decreases in pAVP can occur within a normal range of pAVP in anaesthetized and unanaesthetized animals. A stimulus which is localised to the left atrial receptors also causes a decrease in pAVP and it is likely that this is due to stimulation of the complex unencapsulated endings in the atrium, with myelinated afferent fibres. Evidence is lacking that changes in the stimulus to ventricular receptors or to cardio-pulmonary receptors with C-fibre afferents influences pAVP. The diuretic response to left atrial distension is two-fold, an increase in free water clearance and a natriuresis. The increase in free water clearance is due to the decrease in pAVP; the cause of the natriuresis is unknown. The changes in pAVP occur rapidly in response to atrial distension (within 5 min). The stimulus provided to atrial receptors by atrial distension and the decrease in pAVP is maintained for at least 90 min. pAVP is also modulated in response to small changes in blood volume (+/- 10%). The changes in pAVP that occur over this range of blood volume are likely to be in the range of 1-10 pg/ml and to have their effects on renal water excretion rather than on vascular resistance. The much larger changes in pAVP which occur with greater degrees of blood loss, and which can affect vascular resistance are likely to be produced by changes in the stimulus to other receptors, but a low input from atrial receptors may be permissive for these stimuli to be effective. More work is needed to clarify the relationship between inputs from different receptor types.

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[1]Supported by grants from the Medical Research Council of Canada and the B.C. Heart Foundation.

0024-3205/85 $3.00 + .00

The hypothesis that the circulatory blood volume was under the control of a regulatory mechanism was first expressed, in its simplest form, by Peters (1) who stated that "the fullness of the blood stream may provoke a diuretic response on the part of the kidney". Gauer and Henry (2) expanded the concept that volume regulation is an integral part of over-all cardiovascular regulation and emphasized that with small changes in volume (+/- 10%) responses were most likely to involve the baroreceptors of the low pressure system in the great veins and the heart. The experiments of Henry, Gauer and Reeves (3) had previously demonstrated that mitral obstruction was associated with a diuresis, and Henry and Pearce (4) provided evidence that mitral obstruction stimulated cardiac atrial stretch receptors with afferent fibres in the vagus nerves. Gauer and Henry (2) suggested that the diuretic response to left atrial distension was due to a change in the secretion of antidiuretic hormone (arginine vasopressin, AVP) and/or renal haemodynamics. Some reviewers have emphasized the possible importance of changes in plasma AVP concentration (pAVP) in cardiovascular regulation (5). Others have seriously questioned the hypothesis that stimulation of atrial receptors causes a decrease in pAVP (6,7) and that the decrease in pAVP is then wholly or partially responsible for a diuresis. The recent availability of sensitive radioimmunoassays (RIA) for AVP and the development of techniques for localising a stimulus to left atrial receptors have allowed a number of conclusions to be reached. This review will not reassess work which has been discussed in detail in a number of previous reviews (2,6,7), but will concentrate on recent work which examines the relationship between atrial receptors, pAVP and volume control in the dog.

1. The Relationship Between Mitral Obstruction and Plasma AVP.

The experiments of Henry et al. (3) and Henry and Pearce (4) provided only indirect evidence that mitral obstruction caused a decrease in pAVP. This hypothesis was based not on direct measurements of pAVP but on the characteristics of the diuretic response, namely that the urine became more dilute and that there was a delay of 5-10 min after the start of atrial distension before the increase in urine flow began. This time course was consistent with the information available at that time, that the half-life of AVP in the plasma was about 6 min (8). Early attempts to measure pAVP used a bioassay and showed a reduction in pAVP during mitral obstruction (9,10,11,12,13). These experiments have been criticized (6) because of variable protocols and because of high levels of pAVP, which were well above the range of 1-10 pg/ml found in normally hydrated dogs (14) and which were unlikely to be associated with changes in urinary concentration (15). The only series of experiments which failed to show a decrease in pAVP during mitral obstruction were those of Kappagoda et al. (16) and it is likely that the bioassay used in those experiments was not sufficiently sensitive to detect the changes in pAVP (17).

More recently investigators have used RIA to measure pAVP. A decrease in pAVP was shown by de Torrente et al. (18) during atrial distension but the average pAVP during atrial distension was 12.3 pg/ml, a value greater than that normally

associated with maximal urinary concentration. Zucker et al. (19) also showed a significant decrease in pAVP during mitral obstruction and in these experiments pAVP appeared from their diagram to change from about 10 pg/ml to 5 pg/ml. No attempt was made to correlate the changes in pAVP to changes in urinary excretion in individual experiments. Recently, using anaesthetized dogs in which pAVP was between 2-8 pg/ml we (20) sampled blood at 2 min intervals and showed that mitral obstruction led to a rapid decline in pAVP, a steady lower value being reached in 4 min. There was an equally rapid increase in pAVP after removal of the mitral obstruction. The rapid decline in pAVP with mitral obstruction was consistent with the data of Weitzman and Fisher (15) which showed that the disappearance of AVP from the blood could be described by a two component system, one with a time constant indicating a half life of 1.4 min, the other giving a half life of 4.1 min. We (20) also showed that cooling the cervical vagus nerves to 8-10°C, a temperature which blocks the increases in the frequency of discharge in fibres from atrial receptors during atrial distension, prevented the decrease in pAVP associated with mitral obstruction. In fact vagal cooling caused a small increase in baseline pAVP and there was a further small increase in pAVP during atrial distension. Bennett et al. (17,21) also used anaesthetized dogs with pAVP in the same range and confirmed that mitral obstruction for 30 min (samples were taken every 10 min and pooled before assay) caused a significant decrease in pAVP. They also showed that cooling the vagus nerves to 9°C prevented the decrease in pAVP during mitral obstruction. They did not observe an increase in baseline pAVP during vagal cooling. Their suggestion (21) that the increase in pAVP during vagal cooling which we observed (20), was secondary to haemodynamic changes is unreasonable, since in our experiments vagal cooling caused an increase in mean arterial pressure which, if anything, should cause a decrease in pAVP.

In unanaesthetized dogs with pAVP within the normal range, Fater et al. (22) and Schultz et al. (23) showed that mitral obstruction caused a significant decrease in pAVP. The decrease in pAVP during mitral obstruction did not occur in dogs which had undergone previous cardiac denervation. Baseline pAVP was not different between cardiac denervated and sham-operated dogs. These findings were confirmed by Kaczmarczyk et al. (24), who found significant decreases in pAVP during mitral obstruction in unanaesthetized dogs maintained on either high or low sodium diets. In both series pAVP was within the normal range (1-10 pg/ml).

There can now be no doubt that mitral obstruction causes a significant decrease in pAVP in both unanaesthetized and anaesthetized dogs either when pAVP is elevated or when it is within the normal range. The fact that cooling the vagus nerves to a temperature which blocks increases in activity in myelinated afferents, prevents the decrease in pAVP, suggests that stimulation of atrial receptors may be the afferent mechanism by which the decrease in pAVP is brought about. But cooling to 9°C decreases activity in a minority of non-myelinated afferents (35) and mitral obstruction provides a stimulus which is not limited to the left atrium; there is an increase in

pressure in the pulmonary vascular bed, an increase in heart rate and a decrease in mean right atrial pressure (23). In conscious dogs mean arterial pressure increases (24,25) and in anaesthetized dogs it decreases (3,26). Thus the discharge from a large number of vascular receptors may be altered and the effects of mitral obstruction cannot be attributed solely to stimulation of left atrial receptors.

2. The Relationship Between Stimulation of Left Atrial Receptors and Plasma AVP.

In their initial experiments, Henry et al. (3) showed that a diuretic response occurred during mitral obstruction, but did not occur when pulmonary arterial pressure was increased by the same amount by snaring the pulmonary veins or by embolization of the pulmonary vascular bed. Schultz et al. (23) used a somewhat similar protocol in conscious dogs. The intrathoracic circulation was obstructed at five sites; the mitral valve, the pulmonary veins, the main pulmonary artery, the tricuspid valve and the inferior vena cava. Only obstruction of the mitral valve caused a decrease in pAVP thus supporting the observations of Henry et al. (3), that the stimulus causing a decrease in pAVP was likely to be within the left atrium. To provide a localised stimulus to left atrial receptors, we (27) devised a technique for placing small balloons in the intrapericardial portions of the pulmonary veins on one side of the left atrium, whilst allowing blood to flow unimpeded through the pulmonary veins on the other side of atrium. Since the majority of the left atrial receptors lie close to the pulmonary vein-atrial junctions inflation of these balloons caused marked stimulation of the atrial receptors (28) comparable to that produced by increasing left atrial pressure. Stimulation of the left atrial receptors by this means causes a reflex increase in heart rate (27), a decrease in renal vascular resistance (29), a decrease in activity in sympathetic nerve fibres to the kidney (30) and a dilute diuresis (31) but does not produce significant changes in mean arterial pressure (27). Using this technique of localised stimulation of atrial receptors, we have recently shown a significant decrease in pAVP in anaesthetized dogs during stimulation (32) and this has been confirmed by Bennett et al. (17). In both series pAVP was within the normal range. Cooling the vagus nerves to 8-10°C prevented the decrease in pAVP during stimulation of atrial receptors (32) and caused a small increase in pAVP during the control periods as previously reported (20).

Pulmonary vein distension causes a significant increase in heart rate and this change could have altered the stimulus to vascular receptors other than those in the left atrium. This reflex increase in heart rate was abolished by vagal cooling (8-10°C); as was the increase in pAVP. When the reflex increase in heart rate was prevented by administration of propranolol, with the vagus nerves intact, pulmonary vein distension still caused a significant decrease in pAVP (32). These experiments provide strong evidence that a stimulus which is localised to the left atrial receptors causes a decrease in pAVP. Before concluding that the left atrial receptors form the afferent limb of a reflex arc which decreases pAVP in response to an increase in atrial pressure and by inference,

increases pAVP when atrial pressure decreases, it should be noted that the experiments described have shown only decreases in pAVP in response to increased stimulation of the receptors and a return to previous values of pAVP on removal of the stimulus. Goetz et al. (33) were unable to demonstrate an increase in pAVP (bioassay) when mean atrial transmural pressure was decreased by increasing pressure in a pericardial pouch constructed around the atria. More recently Goetz et al. (34) showed that infusing lidocaine into a pericardial pouch around the atria abolished impulses from atrial receptors in anaesthetized dogs but did not cause any change in pAVP (RIA) in unanaesthetized dogs. These findings suggest that normal atrial receptor activity has only a minor effect on AVP secretion. However it should be noted that vagal cooling to 8-10°C has the effect of reducing the maximum frequency of transmission of impulses and, depending on the initial activity of the receptor, may have little effect on the baseline activity conducted from the receptor (35). It does also lead to a marked reduction in the transmission of the high frequency systolic burst of impulses in aortic baroreceptor fibres (Ledsome, unpublished).

Although local distension of the pulmonary vein-left atrial junctions may stimulate some receptors with non-myelinated afferents the majority of these are not blocked at 8-10°C (35). It may be concluded that a stimulus localized to the left atrial receptors, with myelinated afferent fibres, causes a significant decrease in pAVP and that the afferent path for this reflex response lies in the vagus nerves. There is no evidence available which indicates that removal of the normal tonic input from the atrial receptors is necessarily associated with a large increase in pAVP in the absence of changes to the input from other vascular receptors.

3. Intrathoracic Receptors Other than Left Atrial Receptors and Plasma AVP.

In many discussions of the reflex control of pAVP the effects of changing the stimulus to arterial baroreceptors are compared to the effects of a presumed change in the stimulus to "cardiopulmonary receptors". There is an implication that a number of receptor types situated in the cardiopulmonary area may influence pAVP (e.g. 37). The experiments of Schultz et al. (23) suggest that the stimulus provided by mitral obstruction leads to a decrease in pAVP by acting on receptors in the left atrium and not on other receptors in the pulmonary vascular bed. Whether stimulation of right atrial receptors influences pAVP is not known. It has been shown that right atrial stretch inhibits activity in neurosecretory units in the supraoptic nucleus and may decrease pAVP (36), but the stimulus used may not have been specific for right atrial receptors and pAVP remained decreased after removal of the stimulus casting doubt on the significance of the response. Localized stimulation of right atrial receptors leads to the same reflex changes in heart rate (38) and urinary excretion (39) observed with stimulation of left atrial receptors. Schultz et al. (23) did not observe a decrease in pAVP during obstruction of the tricuspid valve, but right atrial pressure was increased by only

3 mmHg and was accompanied by a decrease in left atrial pressure of 2 mmHg. Thus although it seems likely that stimulation of right atrial receptors will reduce pAVP as does stimulation of left atrial receptors, direct evidence for this is still lacking.

It has been suggested that receptors in the ventricles may influence pAVP. Thames et al. (40) showed that intracoronary injection of cryptenamine (a veratrum alkaloid) inhibited the increase in pAVP seen after haemorrhage in sino-aortic denervated dogs and attributed this effect to stimulation of left ventricular receptors. They were unable to eliminate the possibility that the cryptenamine also stimulated left atrial receptors. These experiments were repeated in conscious dogs by Zucker et al. (41) who showed that intracoronary injection of veratrine prevented the rise in pAVP induced by hypotension but was not capable of reducing pAVP below basal levels. Methylene blue staining indicated their injections were probably localised mainly to the posterior wall of the left ventricle. The possibility remains that the stimulation of ventricular receptors by veratrine may influence pAVP, but the nature and "normal adequate stimulus" of the receptors involved is not defined. There is no direct evidence that any "cardiopulmonary receptors" other than atrial receptors influence pAVP. The term cardiopulmonary receptors is often misleading and should be reserved, as suggested by Thoren (42), to refer to non-myelinated (C-fibre) afferents from the heart and lungs. The C-fibre afferents from these different regions appear to have common functions and can be reasonably grouped whereas the myelinated afferents from the atria have very different and specific reflex functions compared to other myelinated afferents.

It is known that carotid arterial baroreceptors may modulate pAVP (10,43). However, there is frequently little change in pAVP when the carotid sinus and aortic nerves are sectioned (44) provided the vagus nerves are intact. Section of the aortic and vagus nerves with the carotid sinus nerves intact causes a significant rise in pAVP (44) and vagal cold block (0°C) in aortic denervated dogs was said to cause a "large" increase in pAVP (37). There was in fact an approximate doubling of pAVP on vagal cold block when carotid sinus pressure was held constant (37). The large absolute change in pAVP (from 200 pg/ml to 400 pg/ml) may have been a reflection of the very high baseline levels of pAVP in the preparation. When carotid sinus pressure was increased from 50-200 mmHg during vagal cold block there was a decrease in pAVP (37). These experiments indicate a complex interaction between arterial baroreceptors and receptors with afferents in the vagus nerves, most probably atrial receptors, but the nature of the interaction is insufficiently defined at present. Experiments to examine this problem will have to be done with pAVP within a more normal range and with the interaction studied in more detail.

4. Atrial Receptors, Plasma AVP and Diuresis.

Mitral obstruction (3) and localised distension of the pulmonary vein-left atrial junctions (31) cause a dilute

diuresis. It is now clear, as discussed above, that mitral obstruction and pulmonary vein distension cause stimulation of left atrial receptors and a reduction in pAVP. However, this does not necessarily establish a cause and effect relationship between the changes in pAVP and the diuretic response. To demonstrate that the dilute diuresis induced by stimulation of atrial receptors is due to the decrease in pAVP it must be shown that there is a consistent relationship between urinary concentration and pAVP during the diuresis and these changes must be observed over a range of pAVP which is known to be capable of eliciting changes in urinary concentration in the experimental preparation. There have been a number of problems in establishing this relationship in earlier studies. The time consuming nature of the assays for AVP and the relatively low sensitivity of the bioassay together with the need for blood samples of the order of 10 ml have reduced the number of samples taken for pAVP measurement. Most frequently one sample has been taken before, one during and one after the period of atrial distension (12,18,24). Since pAVP may fluctuate markedly within even a 10 min period and pAVP may show episodic changes especially during states of increased pAVP (45), a single sample may not be representative of the average pAVP during the observation of a diuretic response lasting 30-60 min. In addition, during atrial distension, the urine flow and osmolality do not reach a steady state to which the average pAVP can be related (see below). Frequent samples were taken by Shu'ayb et al. (11) and were replaced with blood. Unfortunately their results were presented as a series of examples from which it appeared that there was not always a decrease in pAVP with atrial distension and there was sometimes an increase in urine volume without a change in pAVP; urine osmolality was not given.

The relationship between pAVP and urine volume and osmolality was recently examined (24) in conscious dogs in which mitral obstruction was performed for 60 min. A single sample of blood was taken 30 min before mitral obstruction, at the mid point of the obstruction and 30 min after removal of the obstruction. The pAVP values varied between 2.3-6.0 pg/ml, decreased in every experiment, and were clearly within the range which would be expected to cause changes in urinary concentration in conscious dogs. However, no relationship was found between the pAVP values and the urine volume and osmolality during the 60 min periods. Bennett et al. (21) used anaesthetized dogs in which the pAVP during the 30 min control and experimental periods was measured on three samples taken at 10 min intervals and pooled before measurement. The pAVP during the control periods was 4.0 pg/ml and decreased to 1.6 pg/ml during atrial distension, but (21) there was no significant correlation between the change in urine flow and either the percentage decrease or absolute decrease in pAVP. This is not surprising since the relationship between urine osmolality and pAVP is non-linear and much more dependant on the absolute value of pAVP than on the magnitude of changes. Recently, also using anaesthetized dogs, we (46) have examined the relationship between the time course of the changes in pAVP and urinary concentration during a 30 min period of mitral obstruction. Urine was collected at 10 min intervals and pAVP was measured

at the mid point of each collection period. It was found that pAVP was decreased 5 min after the start of atrial distension but that urine osmolality and volume did not change until 10-20 min after the start of atrial distension. After removal of the atrial distension pAVP was increased within 5 min, at a time when urine osmolality was at its minimum. Because of the differences in the time course of the changes, we compared pAVP with urine osmolality in the urine collected 15 min after the plasma sample. Although there was a wide variation in the relationship between pAVP and urine osmolality between animals, as has been reported in conscious animals (15), there was a significant correlation ($P < 0.05$) between the average pAVP in each 10 min period in the 12 experiments and the average urine osmolality in the following period. The average pAVP during the control periods was 6.5 pg/ml and pAVP decreased to 3.3 pg/ml during atrial distension. These results met the criteria suggested by Linden and Kappagoda (6) to establish a causal relationship between the changes in pAVP and the diuresis: pAVP decreased with each atrial distension, and changes in urine concentration and volume occurred only when pAVP decreased to values known to be associated with changes in urinary concentration. In one test when pAVP was high in the control periods (30 pg/ml), despite a large change in pAVP, (to 10 pg/ml), there was little change in urine osmolality. In other tests there were relatively small changes in both pAVP and urine osmolality. Urine osmolality decreased to less than that of plasma in three experiments and in each of these pAVP decreased to 2.0 pg/ml during the period of atrial distension. The relationship between pAVP and plasma osmolality in these experiments was similar to that described by Quillen and Cowley (47) in conscious dogs and was altered by atrial distension in the same manner that volume expansion altered the relationship in their experiments (47).

The relationship between changes in pAVP and the diuretic response to atrial distension has been assessed by examining the effects of infusion of exogenous AVP during atrial distension. Some confusion has arisen because of early difficulties in assessing the effective dose of AVP. In conscious dogs undergoing water diuresis Shannon (54) and Verney (55) demonstrated maximum decreases in urine flow with doses of AVP of 0.01 mU/kg/min. On the basis of these observations, we (26) infused doses of AVP of 0.025 and 0.1 mU/kg/min and found that there was still an increase in urine volume in response to atrial distension. A similar dose of AVP was used by Lydtin and Hamilton (25) in conscious dogs, they found that the diuretic response was not eliminated but that in prehydrated animals the size of the response was markedly reduced. In more detailed experiments, we (56) infused five different doses of AVP and found that doses of AVP greater than 0.1 mU/kg/min were needed to provide maximally effective antidiuresis and urinary concentration under the conditions of our experiments. We also found (56) that although infusion of AVP could prevent increases in free water clearance associated with atrial distension it did not prevent the small changes in solute excretion. These findings were confirmed by Gillespie et al. (57) who infused AVP at a rate of 0.025 mU/kg/min and also gave an intramuscular loading dose of AVP at the start of the experiments. The

effectiveness of AVP in concentrating the urine is known to be dependant upon osmolal clearance (58) and upon the state of hydration (59,60,61). Since higher infusion rates than expected were needed to prevent changes in free water clearance and because a pAVP of 10 pg/ml is associated with maximal urinary concentration in conscious dogs (14) it has been suggested (6) that the relationship between pAVP and urine osmolality may be different in the anaesthetized as compared to the unanaesthetized dog (48). There is no evidence that this is so and our results (46) provide evidence that there are no differences, at least with chloralose anaesthesia. It is widely believed (e.g. 10) that when studies are carried out on anaesthetized dogs subjected to surgery, such as thoracotomy, there will be high basal levels of pAVP. However, anaesthesia with many common anaesthetics does not of itself cause increases in pAVP (14) and surgical trauma, not associated with blood loss does not cause an increase in pAVP (49,50,51,52). Provided care is taken during anaesthesia and the surgical preparation so that baseline pAVP is within the normal range (20) then the anaesthetized preparation shows changes in pAVP in response to atrial distension which are similar to those in unanaesthetized dogs. The changes in pAVP are also likely to produce changes in urinary concentration which are similar in anaesthetized and unanaesthetized dogs (46). Thus although there may be differences in the neurohumoral control of the circulation after anaesthesia (53) these are not sufficiently great to preclude the use of anaesthetized preparations to study the control of release of AVP at normal pAVP or examination of the effects of AVP on renal function.

Recent experiments using RIA for pAVP have allowed a more accurate assessment of the relationships between infusion rates, pAVP and urinary concentration. Using water loaded conscious dogs in which endogenous release of AVP was suppressed, Weitzman and Fisher (15) found that maximum urine osmolality was not achieved until the infusion rate was greater than 0.136 mU/kg/min and pAVP was approximately 10 pg/ml. Similar results were obtained by Montani et al. (62), also using conscious dogs. These findings are consistent with our earlier results (56) showing that infusion at a rate of 0.1 mU/kg/min was needed to prevent changes in free water clearance in anaesthetized dogs. Kaczmarczyk et al. (24), showed in conscious dogs, that infusion of AVP at 0.05 mU/kg/min, begun 20 min before atrial distension, caused an increase in urine osmolality during the period of atrial distension. From the data of Weitzman and Fisher (15) and Montani et al. (62) an infusion at 0.05 mU/kg/min would be expected to increase pAVP slightly more than atrial distension would lower it. There is therefore no support for the hypothesis that there is a difference in the sensitivity of the kidneys to AVP between anaesthetized and unanaesthetized dogs. The findings from experiments involving infusion of exogenous AVP are all consistent with the hypothesis that the increase in free water clearance, associated with stimulation of atrial receptors is due to the decrease in pAVP.

In addition to an increase in free water clearance, atrial distension causes an increase in sodium excretion which is unaffected by pAVP. The increase in sodium excretion is small

and inconsistent in anaesthetized animals (6) but the mechanisms involved in the increase in sodium excretion in unanaesthetized dogs have been studied in detail (63,64,65,66, 67). Despite this careful and extensive work at present the mechanism causing the natriuresis is unexplained, but it is clear that a blood borne agent acts on the kidney and that this agent is not AVP. The mechanism producing the natriuresis remains of great interest since for long term volume control a mechanism producing only excretion of water is inadequate. It would rapidly lead to an increase in plasma osmolality which would limit water loss by increasing pAVP. Satisfactory volume control requires a mechanism for controlling both water and solute excretion. The recent demonstration of a powerful natriuretic factor present in the atrial myocardium (68) has excited much interest. It is possible that such a natriuretic factor could be released either by local distension of the atria acting directly to promote release, or secondarily to stimulation of atrial receptors with an efferent neural or humoral stimulus acting on the heart, causing release. In anaesthetized dogs either vagal section (26) or injection of a local anaesthetic into the pericardial sac (31) completely prevented the natriuresis associated with atrial distension. In unanaesthetized dogs either cardiac denervation (66) or a local anaesthetic applied to the atria (34) prevented the increase in sodium excretion associated with mitral obstruction. These findings suggest that the natriuresis induced by atrial distension requires intact cardiac nerves and is therefore not due to local stretch of the atrial wall. The natriuresis associated with stimulation of left atrial receptors by distension of the pulmonary vein-left atrial junctions, was prevented by renal denervation (75) suggesting that stimulation of atrial receptors does not release a natriuretic factor. Elucidation of the possible role of the natriuretic peptide in the control of sodium excretion awaits the development of methods for measurement of concentrations of the peptide in body fluids.

5. The Time Course of the Response to Atrial Distension.

In most experiments the effects of atrial distension have been examined using a 30 min period of atrial distension. If the distension is maintained for a longer period, then the increase in urine volume tends to reach a maximum in 20-50 min and then declines (3,25,26,69). The apparently transient nature of the response has led some authors to question the physiological significance of the response in the long term control of blood volume (25). A transient response could be due to adaptation of the receptors in the atrium (70) and/or to a return of pAVP to control values despite maintained stimulus to the receptors. We examined in detail the time course of the diuretic response (69) and found that although urine volume reached a peak at about 40 min and then showed a decline, urine osmolality and free water clearance remained significantly different from control values 90 min after the start of atrial distension. More recently we (71) reported that pAVP remained decreased throughout the whole 90 min of atrial distension. In these experiments, although urine volume and free water clearance reached a peak at 50 min and there was then a decline it was not possible to show statistically that the decline was

significant. In individual experiments there was an obvious decline in free water clearance after the peak had been reached but even in those experiments pAVP remained decreased and at a constant level during atrial distension.

The fact that pAVP remained decreased suggests that the receptors signalling atrial distension do not adapt to the stimulus during a 90 min distension period. This finding is at variance with the report by Kappagoda and Padsha (70) that the ability of atrial receptors to transduce changes in atrial pressure was impaired after 60 min of increased atrial pressure. However, these authors tested the response to atrial pressure only before and after 60 min of atrial distension and provided no information on how rapidly the change in transducing properties occurred. If the adaptation to a new level of sensitivity was rapid, then since pAVP in our experiments was measured at 10 min intervals (71) the rapid adaptation of the receptors would not be apparent from the measurements of pAVP. Surprisingly, Kappagoda and Padsha (70) did not record atrial receptor discharge during the period of atrial distension. Slowly adapting receptors in the superior vena cava of the rat respond to a maintained increase in pressure with an initial high frequency burst of impulses followed by adaptation in two phases: a rapid decline occurring within 1 or 2 sec followed by a slower decline which is complete within 5 min (72). There was little further change between 5 and 15 min. We found a similar pattern of adaptation in left atrial receptors in the normally beating dog heart (73). Adaptation was found to be complete within 6 min and there was no further decline up to 60 min. Thus although atrial receptors show rapid adaptation they are still capable of signalling an increased atrial pressure for periods of at least 60 min. These findings indicate that the apparent decline of the renal response to atrial distension is not due either to gradual adaptation of the atrial receptors or to a return of pAVP towards control values. There is evidence that when atrial distension is maintained for several weeks there is a decrease in atrial receptor activity at any given pressure (19,76).

That the pattern of the urinary response may be determined by the response of the kidneys to pAVP is suggested by our earlier experiments (74) which showed that in anaesthetized dogs decreasing the rate of infusion of AVP from 0.4 mU/kg/min to 0.04 mU/kg/min caused an increase in urine flow and free water clearance which reached a peak and then declined. From information now available (15,62) it appears that infusions at these rates would give pAVP of about 50 pg/ml and 5 pg/ml respectively. Since pAVP was not measured, these results should be interpreted with caution. They do however, raise the possibility that with a change to a new steady level of pAVP there is not necessarily a direct change to a new steady urine osmolality.

6. Atrial Receptors and Blood Volume.

The hypothesis that small changes in blood volume may be associated with changes in pAVP is supported by the results of Share (77), Claybaugh and Share (78), Shade and Share (79) and

by Wang et al. (80) all of whom showed that reductions in blood volume of less than 10% were associated with increases in pAVP. The much larger increases in pAVP reported by these authors with larger decreases in blood volume may be due to changes in the input from receptors other than atrial receptors. However, some authors consider that the great increase in pAVP which occurs with moderate to severe haemorrhage is due to cardiac receptors (81). The latter view is supported by the observation that either vagotomy (77) or cardiac denervation (80) markedly attenuated the increase in pAVP which occurred with losses of 10 - 30% of the blood volume, although both vagotomy and cardiac denervation were without effect on the prehaemorrhage pAVP. It has been shown recently, that in the conscious dog, acute bilateral vagal cold block to 0°C (82) causes only an approximate doubling of pAVP. The relatively minor effect of vagal blockade on pAVP (20,21,77,82) could be partly explained by the fact that vagal block produces an increase in mean arterial pressure. This view receives support from the observation (82) that after chronic sino-aortic denervation, vagal cold block produced a much larger increase (8 times) in pAVP; but even then, the levels reached did not approach those seen in moderate or severe haemorrhage. These findings could be interpreted to suggest that a part of the stimulus contributing to the very large increases in pAVP with haemorrhage could be increased stimulation of receptors with vagal afferents (possibly chemoreceptors 2,85) which cause an active release of AVP. The work of Gupta et al. (76) showed that atrial receptor activity decreased by 50% with a 10% blood loss and to 20% of control values with a 30% blood loss. Expressing the changes as percentages is misleading since control rates of atrial receptor discharge at normal blood volume are of the order of 5-10 impulses/sec. Thus a 30% blood loss involves a decrease of 4-8 impulses/sec. This may be contrasted with the increase of 40-50 impulses/sec which occurs with volume expansion of 25% of blood volume (Ledsome and McFetridge, unpublished). Other evidence also suggests that atrial receptors may have more importance in responses to increases in blood volume than decreases in blood volume. Recent analysis of the relative roles of cardiac and arterial baroreceptor reflexes in conscious rabbits indicated that whereas both reflexes were involved in responses to increasing blood volume, the cardiac receptor reflexes had no effect during blood volume depletion (86). These experiments also emphasized that the interaction between effects from cardiac receptors and arterial baroreceptors was not simply additive. The nature of the interaction between impulses from atrial receptors and those from other receptors in modulating the release of AVP is unknown but it may be that a low input from atrial receptors is necessary for marked increases in pAVP to occur in response to a change in the stimulus to other receptors. More work is needed to precisely define the relationship between the input from the arterial baroreceptors and atrial receptors, preferably in preparations in which pAVP is within the normal range. Discussion of work indicating that in primates and humans, small changes in blood volume do not affect pAVP (84,85) is outside the scope of this review.

In conclusion, there exists, in the dog, a mechanism by which pAVP changes in response to small increases or decreases (+/- 10%) in blood volume. The atrial receptors provide a mechanism by which the changes in pAVP may be brought about. The atrial receptors are likely to be more effective in reducing pAVP in response to increases in blood volume than they are in producing the large increases in pAVP associated with large decreases in blood volume. The decreases in pAVP which occur with stimulation of atrial receptors can occur at normal levels of pAVP and result in changes in the excretion of water by the kidney. This provides one mechanism by which blood volume and body fluid volume may be regulated. However, this mechanism would be self limiting and for efficient regulation of volume a mechanism which also influences sodium excretion is required. Since atrial distension does cause a natriuresis and since the atrial myocytes are now known to contain a natriuretic factor, new information, about the possible role of the atria in the regulation of sodium excretion, may be expected in the near future and should be of great interest.

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Release of atrial natriuretic peptide by atrial distension

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Received February 19, 1985

LEDSOME, J. R., N. WILSON, C. A. COURNEYA, and A. J. RANKIN. 1985. Release of atrial natriuretic peptide by atrial distension. Can. J. Physiol. Pharmacol. **63**: 739–742.

A heterologous radioimmunoassay was used to measure the concentration of immunoreactive atrial natriuretic peptide (iANP) in plasma from the femoral artery of eight chloralose anaesthetized dogs. Mitral obstruction which increased left atrial pressure by 11 cmH_2O increased plasma iANP from 97 ± 10.3 (mean ± SE) to 135 ± 14.3 pg/mL. Pulmonary vein distension increased heart rate but did not increase plasma iANP. Bilateral cervical vagotomy and administration of atenolol (2 mg/kg) did not prevent the increase in iANP with mitral obstruction. Samples of blood from the coronary sinus had plasma iANP significantly higher than simultaneous samples from the femoral artery confirming the cardiac origin of the iANP. Release of iANP depends on direct stretch of the atrium rather than on a reflex involving left atrial receptors.

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On a utilisé un radio immunodosage hétérologue pour déterminer la concentration du peptide natriurétique auriculaire immunoréactif (iANP) dans le plasma de l'artère fémorale de huit chiens anesthésiés au chloralose. Une obstruction mitrale qui augmenta la pression auriculaire gauche de 11 cm d'H_2O augmenta le iANP plasmatique de 97 ± 10,3 (moyenne ± EI) à 135 ± 14,3 pg/mL. Une dilatation veineuse pulmonaire augmenta la fréquence cardiaque mais n'augmenta pas le iANP plasmatique. Une vagotomie cervicale bilatérale et l'administration d'aténolol (2 mg/kg) ne prévinrent pas l'augmentation de iANP induite par l'obstruction mitrale. Le iANP plasmatique d'échantillons sanguins du sinus coronaire était significativement plus élevé que celui d'échantillons de l'artère fémorale, ce qui confirme l'origine cardiaque du iANP. La libération du iANP dépend davantage de l'étirement direct de l'oreillette que d'un réflexe impliquant les récepteurs auriculaires gauches.

[Traduit par le journal]

Introduction

The intravenous injection of extracts from mammalian atrial myocardium has been shown to cause natriuresis (de Bold et al. 1981). The natriuretic activity has been attributed to a peptide (atrial natriuretic peptide, ANP) which has been purified, sequenced, and synthesized (Flynn et al. 1983; Kangawa and Matsuo 1984; Atlas et al. 1984). Both the natural and the synthetic peptides have natriuretic and vasorelaxing properties. If ANP is released into the circulation in response to physiological stimuli, then it may play a significant role in fluid volume and blood pressure homeostasis. Left atrial distension, induced by partial obstruction of the mitral orifice is associated with a diuresis and natriuresis in anaesthetized (Ledsome and Mason, 1972) and conscious dogs (Kaczmarczyk et al. 1981). The diuresis depends upon stimulation of left atrial receptors and a reflex inhibition of the release of vasopressin from the neurohypophysis (Ledsome and Wilson 1984). The cause of the natriuresis remains unknown (Kaczmarczyck et al. 1983). We have measured plasma ANP concentration in anaesthetized dogs, using a heterologous radioimmunoassay (RIA). Mitral obstruction was used to provide stretch of the atrial wall and stimulation of atrial receptors; distension of the pulmonary vein–atrial junctions was used to provide stimulation of a significant proportion of the atrial receptors while stretching only a small portion of the atrial wall. The results show that ANP was released from the heart by a direct effect of increased left atrial pressure rather than by a reflex mediated through stimulation of left atrial receptors.

Methods

Radioimmunoassay for iANP

Blood samples from either the femoral artery or coronary sinus were taken into cold (4°C) syringes and were transferred immediately to cold tubes containing heparin and aprotinin (Trasylol, Miles Pharmaceuticals, Rexdale, Ontario; 20 kallikrein inhibitor units (KIU)/mL blood) and centrifuged (× 3000 *g*) at 4°C. Plasma was separated and stored at −20°C for RIA on the following day. RIA used commercially available rabbit anti-α-atrial natriuretic polypeptide serum (Peninsula Labs., Belmont, CA, RAS-8798, lot No. 006107). This antibody showed 100% cross-reactivity with α-human atrial natriuretic peptide, rat atriopeptin III, and rat ANP (Peninsula Labs, specification leaflet, July 26, 1984). Iodinated ANP was prepared from synthetic rat atriopeptin III (Peninsula Labs., code 8799, lot No. 006101) and standards were prepared from the same source. This lot contained 86% peptide; the values given in this paper were not corrected for this difference from 100%. Iodination was done using the chloramine-T method (Morris 1976) and the iodination mixture was purified on a SP-Sephadex G-25 (Pharmacia, lot No. GF-21619) column described for purification of atriopeptins from atrial extracts (Currie et al. 1984). Specific radioactivity of the iodinated peptide was 1 mCi/μg (1 Ci = 37 GBq). Disequilibrium assay at 4°C consisted of preincubation for 36 h, the addition of the tracer (4000 disintegrations per minute per tube), and an additional 24-h incubation period. Separation of bound from free tracer was done using a second antibody (Peninsula Labs., code GARGG-500, lot No. 006025). Under these conditions, 40–50% of the tracer was bound by the anti-ANP serum. One hundred microlitres of standard or plasma were assayed in triplicate, and results were corrected for nonspecific binding of plasma to iodinated peptide in the absence of antiserum. Displacement of 20 and 50% was obtained with 2.33 ± 0.11 and 8.04 ± 0.32 (mean ± SE, n = 22) pg/tube, respectively. The intra- and inter-assay errors were 3.1 ± 0.6% (n = 15) and 5.8 ± 1.4% (n = 12). Interassay error was calculated only on samples from different RIAs started on the same day, as plasma exhibited progressive loss of immunoreactivity on storage. Recovery experiments showed that 4.92 ± 0.22 pg (mean ± SE, n = 6) were recovered when 5 pg of rat atriopeptin III was added to each tube.

Atrial extract (AE) was prepared from canine atria, stored at −20°C for 18 months. The tissue (77 g wet weight) was placed into 300 mL

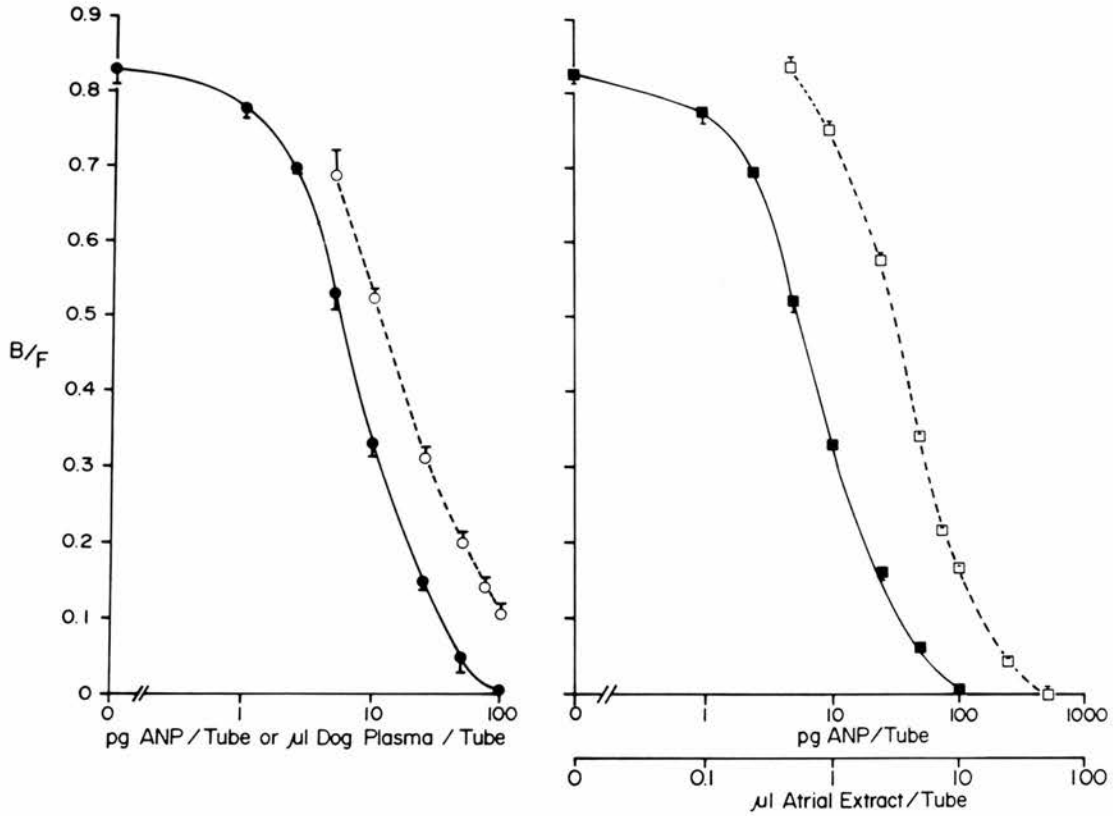

FIG. 1. Assay of immunoreactive ANP. Ordinate: bound−free ratio (B/F) corrected for nonspecific binding. Left panel, abscissa: ANP, in picograms per tube (Atriopeptin III, Peninsula Laboratories Inc., Belmont, CA, U.S.A.) or dog coronary sinus plasma, in microlitres per tube. The high value in coronary sinus plasma was obtained by manual manipulation of the atria during sampling. Solid line: standard curve; broken line: dilutions of plasma. Right panel, abscissa: ANP, in picograms per tube or extract of dog atrium, in microlitres per tube (expressed as 1:300 dilution of the original extract). Solid line: standard curve; broken line: dilutions of atrial extract.

of cold 1 *M* acetic acid, disrupted in an omnimix homogenizer and sonicated two times, 15 s each. Trasylol (Miles, 817113, 10 000 KIU/mL), 0.5 mL, was added to the homogenate. Following centrifugation (4°C, 48 000 *g*, 30 min), the supernate was passed through Whatman Nos. 4 and 52 filters and AE was stored at −20°C.

Experimental protocol

Experiments were carried out on eight mongrel dogs anaesthetized with α-chloralose (100 mg/kg, i.v.). Anaesthesia was maintained with an intravenous infusion of a solution of chloralose (0.5 g/100 mL saline; 2 mL/min). Five animals were prepared with a balloon cannula and a cannula for pressure measurement inserted into the left atrium through the appendage (Ledsome et al. 1983) and small balloons were inserted into each of three pulmonary veins (Wilson and Ledsome 1983). Samples of femoral arterial blood (4 mL) were taken 5 min before and immediately before mitral obstruction, which was induced by inflating the left atrial balloon to increase left atrial pressure by about 11 cmH_2O. Samples were taken 10 and 20 min after the start of atrial distension, the balloon was deflated and sampling was repeated 10 and 20 min after deflation. Distension of the pulmonary veins was induced by injecting 1 mL of saline into each of the three pulmonary vein balloons. Blood samples were taken 10 min before and immediately before distension, 10 and 20 min after the start of distension, and 10 and 20 min after removal of the distension. These tests were repeated after bilateral cervical vagotomy. Mean arterial pressure, heart rate, and mean left atrial pressure were measured at the time of blood sampling. In three other dogs mitral obstruction was performed before and after vagotomy and cardiac beta-receptor blockade with atenolol (Sigma Chemical Co., St. Louis, MO, 2 mg/kg, i.v.). In five dogs a catheter was inserted into the azygos vein and advanced to allow sampling from the coronary sinus.

Results and discussion

Dog atrial extract was prepared to allow demonstration that dog atria contained a high concentration of a substance which showed immunoreactivity to the antiserum used and could be the source of the immunoreactivity in the plasma. The total volume of AE was 260 mL. Absorbance at 280 and 260 n*M* was 34.4 and 39.8 absorbance units, respectively, per millilitre of undiluted AE. RIA of AE (1:1000 dilution) 4 days after extraction revealed the presence of 1400 ± 80 ng of immunoreactive ANP (iANP) per millilitre of undiluted AE. Following a month of storage at −20°C the immunoreactivity decreased to 469 ± 66 ng/mL as shown by three separate RIA experiments. Serial dilutions (1:300 − 1:3000) of AE and dilutions of dog plasma were tested for parallelism in the rat ANP standard curve (Fig. 1). The concentration of iANP in canine atria was thus in the range of 1.7 − 4.7 µg/g of tissue (wet weight). This may be compared with published values for the rat of 5.7 and 13.3 µg/rat, left and right atria, respectively (Gutkowska et al. 1984), obtained using an extraction procedure similar to ours. Higher yields were reported per gram of rat and human atria (Nakao et al. 1984), but these extracts were boiled to neutralize proteolytic activity. Different antibodies and RIA systems were used to arrive at the iANP estimates listed above.

Because the structure of ANP in the dog is unknown and purified dog ANP is not available at present, we were unable to test the antiserum under ideal conditions. However, the parallelism obtained with rat atriopeptin III by dilution of dog plasma and AE (Fig. 1), and the presence of a high concen-

tration of iANP in AE and a significant concentration difference between iANP concentrations in the coronary sinus and peripheral blood (see below), suggest the existence of an immunoreactive substance in the dog which is similar to rat atriopeptin III.

According to the information supplied by the manufacturer, the antiserum shows 100% cross-reactivity with rat ANP, rat atriopeptin III, and α-human atrial natriuretic polypeptide (Peninsula Labs, specification leaflet, July 26, 1984). The fact that it cross-reacts by only 27% with rat atriopeptin II suggests that this antibody is directed towards the C-terminal end of the molecule. Nakao et al. (1984) using an antibody directed towards the common carboxy-terminal fragment of α-human ANP and α-rat ANP also detected immunoreactivity in dog atrial extract.

Mitral obstruction, which caused an increase in left atrial pressure of 11 ± 1.7 cmH_2O, significantly increased ($p < 0.05$) plasma iANP from 97 ± 10.3 to 135 ± 14.3 pg/mL. Heart rate was also increased but there were only small changes in mean arterial pressure (Fig. 2). Pulmonary vein distension caused an increase in heart rate, no change in left atrial pressure or arterial pressure, and a small but not significant increase in plasma iANP. Bilaterial cervical vagotomy did not affect baseline values of plasma iANP and did not prevent the increase in plasma iANP caused by mitral obstruction (Fig. 2). Administration of atenolol (2 mg/kg, i.v.) did not prevent the changes in plasma iANP caused by mitral obstruction. Plasma iANP increased from 68 ± 8 to 119 ± 12 pg/mL ($n = 3$) during mitral obstruction after vagotomy and administration of atenolol. That the iANP was released from the heart was demonstrated by sampling blood directly from the coronary sinus. In 30 of 34 samples in five dogs, taken simultaneously from the coronary sinus and the femoral artery, coronary sinus iANP concentration was higher than that in the femoral artery: 273.2 ± 47.7 vs. 94.4 ± 9.8 pg/mL ($n = 34$). In the two dogs mitral obstruction was tested; mean plasma iANP from the femoral artery was 58 pg/mL in the control periods and 83 pg/mL during mitral obstruction; simultaneous samples from the coronary sinus gave concentration of 98 and 218 pg/mL.

The fact that neither vagotomy nor vagotomy and beta-blockade prevented the increase in plasma iANP in response to mitral obstruction, shows that the release of iANP is likely to be due to local stretch of the atrial wall rather than originating from stimulation of atrial receptors and reflex activation of vagal or sympathetic efferents. This view is supported by the fact that distension of the pulmonary vein–atrial junctions, which provides an effective stimulus to the atrial receptors (Kidd et al. 1978), did not cause a significant change in plasma iANP concentration. These experiments provide direct evidence that the plasma concentration of iANP can be altered by a physiological stimulus and that release from the heart, in vivo, is a direct effect of stretch of the atrial wall. It is not known if the observed changes in plasma iANP concentration were of sufficient magnitude to cause vasorelaxation or natriuresis. Nevertheless, the fact that the heart is capable of releasing this peptide into the plasma, in response to changes in atrial pressure, is likely to be of significance in the control of body fluid volume and possibly in hypertension.

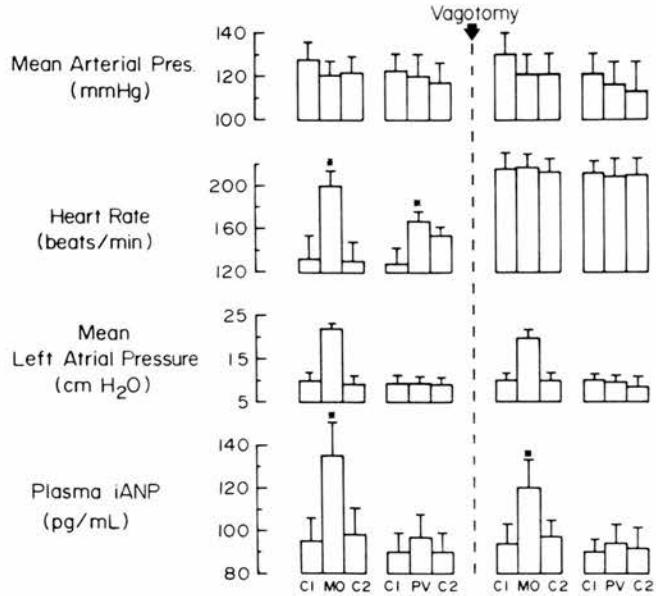

FIG. 2. Mean arterial pressure, heart rate, mean left atrial pressure, and plasma iANP concentration before (C1) and after (C2) mitral obstruction (MO) or pulmonary vein distension (PV). Values are the means of two control and two experimental values in each of five experiments (± SE of mean). Bilateral cervical vagotomy was performed after the first two tests. *, indicate experimental values significantly different from the mean of the control values ($P < 0.05$).

Acknowledgements

This work was supported by grants from the Medical Research Council of Canada and the British Columbia Heart Foundation. The authors are grateful for the assistance of Marie Sweeney and D. Feuchuk.

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Time course of release of atrial natriuretic peptide in the anaesthetized dog

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Received November 13, 1985

LEDSOME, J. R., N. WILSON, A. J. RANKIN, and C. A. COURNEYA. 1986. Time course of release of atrial natriuretic peptide in the anaesthetized dog. Can. J. Physiol. Pharmacol. **64**: 1017–1022.

In 12 chloralose anaesthetized dogs plasma concentration of immunoreactive atrial natriuretic peptide (IR-ANP) was measured using a radioimmunoassay. Plasma IR-ANP was 74 ± 4.8 pg/mL (mean ± SE) and increased by 39 ± 4.1 pg/mL when left atrial pressure was increased by 10 cm H_2O during partial mitral obstruction. Observation of the time course of the changes in IR-ANP during atrial distension showed that IR-ANP was increased within 2 min of atrial distension and declined after atrial distension, with a half-time of 4.5 min. The time course of the changes in IR-ANP was unaffected by vagotomy or administration of atenolol. Maximum electrical stimulation of the right ansa subclavia failed to produce any change in IR-ANP. IR-ANP was higher in coronary sinus plasma than in femoral arterial plasma confirming that the heart was the source of the IR-ANP. The results support the hypothesis that IR-ANP is released from the heart by a direct effect of stretch of the atrial wall rather than by a neural or humoral mechanism involving a reflex from atrial receptors.

LEDSOME, J. R., N. WILSON, A. J. RANKIN et C. A. COURNEYA. 1986. Time course of release of atrial natriuretic peptide in the anaesthetized dog. Can. J. Physiol. Pharmacol. **64**: 1017–1022.

On a déterminé par radioimmunodosage la concentration plasmatique du peptide natriurétique auriculaire immunoréactif (PNA-IR) chez 12 chiens anesthésiés au chloralose. Le PNA-IR plasmatique était de 74 ± 4,8 pg/mL (moyenne ± ET) et augmenta de 39 ± 4,1 pg/mL lorsqu'on éleva la pression auriculaire gauche de 10 cm H_2O durant une obstruction mitrale partielle. L'observation de l'évolution temporelle des variations de PNA-IR durant la distension auriculaire montra que le PNA-IR avait augmenté en moins de 2 min après le début de la distension et qu'il avait ensuite diminué après la distension avec une demi-vie de 4,5 min. Ni une vagotomie ni l'administration d'aténolol n'affectèrent l'évolution temporelle des variations du PNA-IR. La stimulation électrique maximale de l'anse sous-clavière droite ne put provoquer aucune variation du PNA-IR. Le PNA-IR était plus élevé dans le plasma du sinus coronaire que dans celui de l'artère fémorale, ceci confirmant que le coeur était la source du PNA-IR. Les résultats supportent l'hypothèse que le PNA-IR est libéré du coeur davantage par une effet direct de l'étirement de la paroi vasculaire que par un mécanisme neuronal ou humoral impliquant un réflexe des récepteurs auriculaires.
[Traduit par la revue]

Introduction

The myocytes of mammalian atria contain a large number of granules with morphological features similar to those found in peptide-secreting endocrine cells (Jamieson and Palade 1964). Intravenous injections of extracts of atria have been shown to cause a brief but significant diuresis, natriuresis, and hypotension (Ackerman et al. 1984; De Bold et al. 1981; Garcia et al. 1984). Recently, a number of biologically active peptides have been isolated from rat atria, sequenced, and synthesized (Atlas et al. 1984; Currie et al. 1984; Flynn et al. 1983). The peptides have been called cardionatrin (Atlas et al. 1984), atriopeptin (Currie et al. 1984), and atrial natriuretic factor (Seidah et al. 1984); we shall refer to the active peptide as atrial natriuretic peptide (ANP). A similar factor has been isolated, purified, and sequenced from human atrial extracts (α-human atrial natriuretic polypeptide, Kangawa and Matsuo 1984). Recently ANP has been demonstrated to be present in the circulating plasma and the concentration in the plasma has been shown to be increased in rats maintained on a high sodium intake (Tanaka et al. 1984). It appears likely that ANP plays a significant role in body fluid volume and blood pressure homeostasis.

The mechanisms that control release of ANP from the atria have not yet been elucidated nor has it been proven that the atria are the source of circulating ANP. Left atrial distension induced by partial obstruction of the mitral orifice is known to cause a diuresis and a small and inconsistent natriuresis in anesthetized dogs (Ledsome and Mason 1972). In conscious dogs, atrial distension causes a diuresis and a more consistent natriuresis (Kaczmarczyk et al. 1981). The diuresis depends upon stimulation of left atrial receptors and reflex inhibition of the release of vasopressin (AVP) from the neurohypophysis (Ledsome and Wilson 1984). The cause of the natriuresis remains unknown (Kaczmarczyk et al. 1978, 1979, 1983) but could possibly be due to release of ANP during atrial distension. Using a heterologous radioimmunoassay, we have measured the plasma concentration of immunoreactive ANP (IR-ANP) in anaesthetized dogs. To examine the time course of the release of ANP from the atria we used mitral obstruction to provide stretch of the left atrial walls. We have previously shown that stimulation of left atrial receptors by atrial stretch, and activation of a neural reflex, is unlikely to be the mechanism by which atrial distension causes release of ANP from the atrium (Ledsome et al. 1985). The present paper describes the time course of changes in IR-ANP during atrial distension, in plasma from the femoral artery, and from the coronary sinus. We also provide statistical evidence, not previously presented (Ledsome et al. 1985) that neither vagotomy nor the administration of the cardioselective β-blocking agent atenolol affect the release of IR-ANP. In addition, it is shown that IR-ANP is not released by maximal stimulation of the cardiac sympathetic nerves.

Methods

Twelve dogs weighing 16.1 ± 3.6 kg (mean + SD) were given morphine sulphate, 0.5 mg/kg, s.c. One hour later, under local anaesthesia (1% mepivacaine hydrochloride, Winthrop Laboratories, Aurora, Ontario), a saphenous vein was catheterized and anaesthesia was induced with infusion of 10 mL/kg of an α-chloralose solution (BDH Chemicals, U.K.; 1 g/100 mL in 0.9% w/v NaCl). After completion of the surgical procedures, anaesthesia was maintained by the continuous infusion of a chloralose solution (0.5 g/100 mL, 0.9% w/v NaCl) at a rate of 2 mL/min.

As soon as possible after the induction of anaesthesia, tracheotomy was performed and artificial respiration was started with 40% O_2 in N_2, supplied from a respiration pump (Harvard Apparatus Co., MA, model

614); the rate (about 14/min) and stroke volume (about 50 mL/kg body weight) were adjusted to approximately equal that of the animal's spontaneous ventilation. When the chest was opened an expiratory resistance equivalent to 3 cm H_2O was provided. At intervals during the preparation, samples of arterial blood were taken and pH, P_{CO_2}, and P_{O_2} were measured using appropriate electrodes (Corning Glass Works, MA, model 165/2). Adjustments were made to the respiratory pump or, infusions of sodium bicarbonate solution (1 *M*) were given to bring P_{CO_2} to between 35 and 40 mmHg (1 mmHg = 133.322 Pa) and pH within the range 7.3–7.4. No adjustments were made after the experimental period had begun. During the surgical procedures, which lasted about 1 h, 7.7 mL/kg of dexran (Dextran 70 in 0.9% NaCl, Pharmacia Ltd., Quebec) was infused. Oesophageal temperature was maintained at 37 ± 1°C using a heated table and a temperature controller (Yellow Springs Instrument Co., Yellow Springs, OH).

Both vagosympathetic nerves were identified in the neck and loose ligatures placed around them. Both femoral arteries were cannulated, one for the measurement of arterial pressure and one for obtaining samples of arterial blood. In six dogs thoracotomy was performed in the left fifth intercostal space. A balloon made from a 2-cm length of the finger of a surgical glove and 2 mm bore polyethylene tubing was introduced into the left atrium through the atrial appendage together with a 1-mm bore Teflon catheter for the measurement of atrial pressure. The balloon catheter was clamped to prevent movement when the balloon was inflated. In two of these dogs, at the end of the experiment, a right thoracotomy was performed and a cannula inserted through the azygos vein, into the coronary sinus, to obtain samples of coronary sinus blood. In another six dogs a balloon was placed in the left atrium through the appendage, as above, but atrial pressure was measured through a stainless steel cannula, 1.5 mm bore, placed in the middle pulmonary vein. In these six dogs a second thoracotomy was performed through the right third intercostal space, the right ansa subclavia was identified, and a loose ligature was placed around it. A 1.5-mm bore polyethylene catheter was inserted into the azygos vein and placed so that the tip lay within the coronary sinus, about 2 cm proximal to the ostium in the right atrium. Femoral arterial and left atrial pressure were recorded using strain gauges (Statham Instrument Co., Puerto Rico, P23Db) and DC amplification. Mean pressures were obtained electrically using an R-C circuit with a time constant of 2 s. Heart rates were counted from an electrocardiogram obtained from leads on a hindlimb and the chest wall. Recordings were made on a ultraviolet light recorder (Honeywell, Denver, CO., Visicorder, 1608).

Experimental protocol

Samples of venous blood were taken from two dogs before giving morphine and from eight dogs 1 h after giving morphine but before administration of the anaesthetic. A period of 1 h was allowed for stabilization after completion of the surgical procedures. In the first six experiments a recording was then made and a sample of arterial blood was taken (4 mL). Fifteen minutes later a second sample and recordings were taken; subsequent samples and recordings were taken at intervals as shown in Fig. 1. Both vagosympathetic nerves were then cut in the midcervical region and the protocol was repeated. In one of the experiments the vagus nerves were left intact and atenolol (Sigma Chemical Co., St. Louis, MO, 2 mg/kg, i.v.) was administered at the time the vagus nerves would have been cut.

In the second six experiments the same protocol was followed for the first 13 samples as in the first group of experiments, but samples of blood were taken from both the femoral artery (4 mL) and from the coronary sinus (3 mL, samples 3–12, see Fig. 3). This covered the period of the first test of atrial distension. Both vagosympathetic nerves were then cut and a pair of stimulating electrodes, mounted on a lucite block, were placed around the right ansa subclavia. A ligature was tied around the ansa subclavia proximal to the stimulating electrodes. Sampling from the femoral artery and recordings were taken 5 and 10 min later. The right ansa subclavia was then stimulated for 20 min using a Grass stimulator (Grass Inst. MA, model S8) and stimulus isolation unit (Grass Inst., MA, model SIU 1) with the parameters of 10 Hz, 2 ms and 10 V. Sampling and recording were carried out at 2, 5, 10, and 20 min of stimulation and 2, 5, 10, and 20 min after stimulation. In three of the six dogs simultaneous samples from the coronary sinus were taken in the two periods before stimulation, 10 and 20 min after the start of stimulation and 10 and 20 min after stopping stimulation. Atenolol (2 mg/kg) was then given i.v. The effectiveness of the cardiac β-blockade was tested before and after giving the atenolol, by injection of isoproterenol (K & K Labs., Plainview, NY; 0.5 μg/kg) and measurement of the change in heart rate. Adequate blockade was judged to be present if the increase in heart rate on injection of isoproterenol was reduced by 90% from its preblockade value. Sampling and recording were carried out 2, 15, 20, and 25 min after giving the atenolol. A further 1 mg/kg of atenolol was then given and a second test of atrial distension carried out. As before, sampling and recording were done 2, 5, 10, and 20 min after the start of atrial distension and 2, 5, 10, 20, and 30 min after removal of the distension. Samples were not taken from the coronary sinus during this last test.

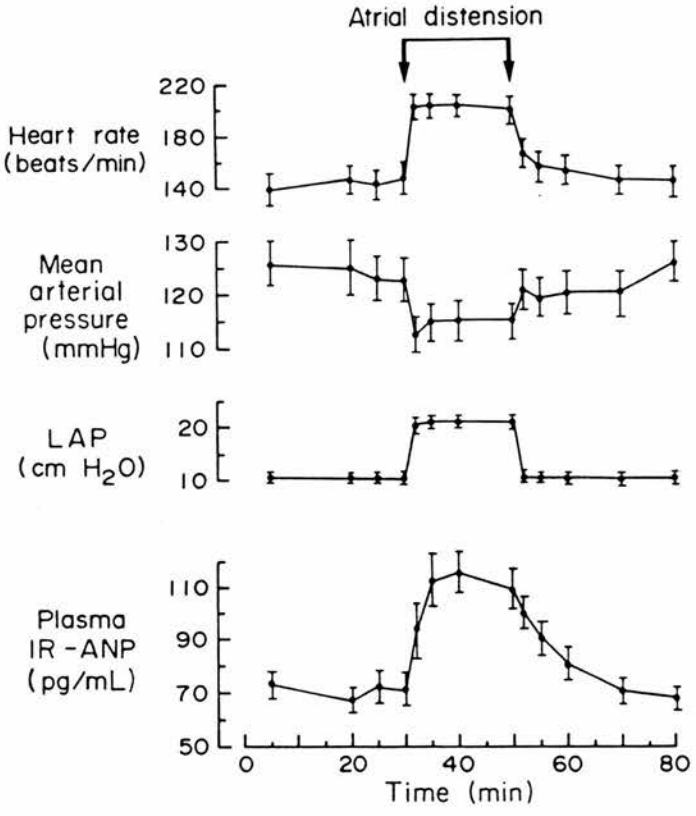

FIG. 1. Effects of left atrial distension, by partial mitral obstruction, on heart rate, mean arterial pressure, mean left atrial pressure (LAP), and immunoreactive atrial natriuretic peptide concentration (IR-ANP) in the plasma. (mean ± SE; n = 12.).

All blood samples were taken in cold (4°C) plastic syringes and transferred immediately to cold EDTA tubes containing heparin and aprotinin (Trasylol, Miles Pharm., Rexdale, Ont., 20 KIU/mL of blood) and centrifuged (× 3000 g) at 4°C. Plasma was separated and stored at −20°C for radioimmunoassay (RIA) the following day. Haematocrit was measured on each sample using a microhaematocrit centrifuge. The volume of blood removed with each sample was immediately replaced with an equal volume of dextran to which, after the first sample, the red blood cells from the previous sample had been added.

Radioimmunoassay

RIA used rabbit anti-α-ANP (atrial natriuretic polypeptide) serum (Peninsula Labs., Belmont, CA, RAS-8798, lot 006107). Details of the assay (Wilson et al. 1986) and its application to dog plasma (Ledsome et al. 1985) have been published previously.

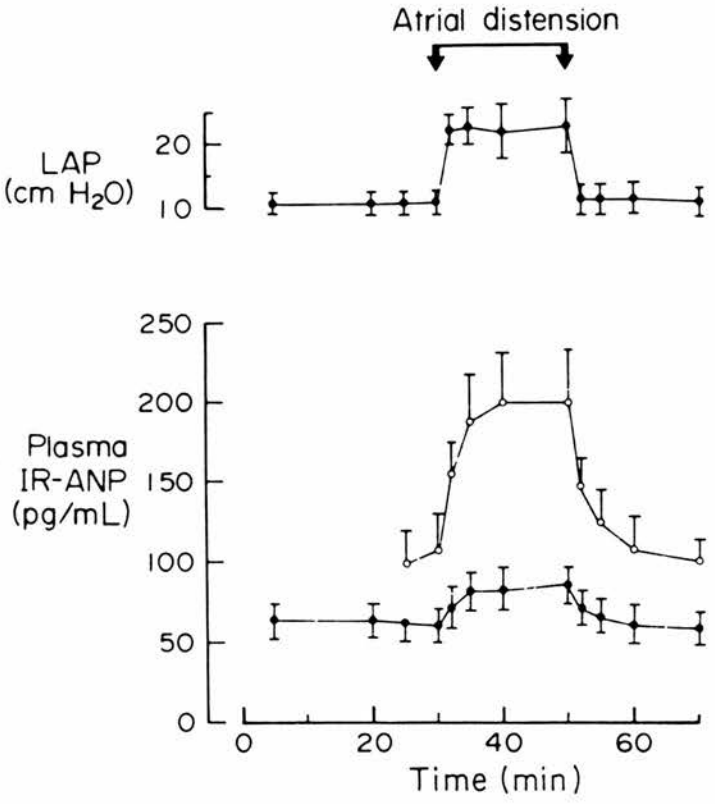

FIG. 3. Changes in left atrial pressure (LAP) during atrial distension and plasma concentration of IR-ANP in blood from the femoral artery and from the coronary sinus. Values in plasma from the femoral artery (●) and values in plasma from the coronary sinus (○). (mean ± SE; $n = 4$.)

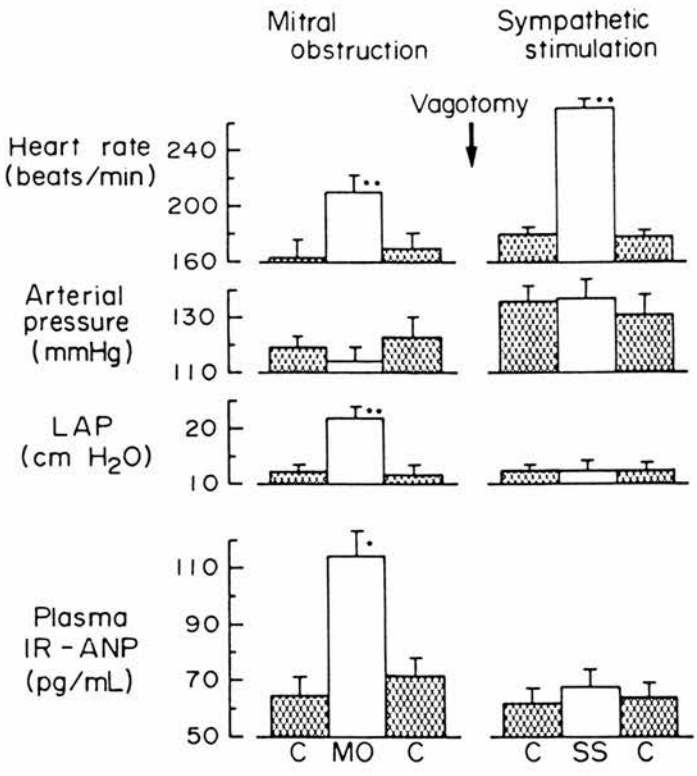

FIG. 4. Effects of left atrial distension by mitral obstruction compared with the effects of stimulating the right ansa subclavia (SS). Bilateral vagotomy was performed before sympathetic stimulation. (mean ± SE; $n = 6$.).

min after removal of the atrial distension, coronary sinus plasma IR-ANP had returned to the control value.

Effects of vagotomy

Bilateral cervical vagotomy was carried out in 11 dogs. Measurements of the variables taken 10 min before and immediately before vagotomy were compared with measurements taken 20 and 25 min after vagotomy (first five dogs), or 5 and 10 min after vagotomy (second six dogs). Since the results were not significantly different between these two groups, the results have been pooled. Vagotomy caused a significant increase in mean arterial pressure from 121 ± 4.5 to 133.5 ± 4.9 mmHg ($p < 0.01$) and a significant increase in heart rate from 159 ± 7.0 to 196 ± 8.3 beats/min ($p < 0.01$). There was no change in mean left atrial pressure which was 10.6 ± 0.9 cm H_2O before vagotomy and 11.1 ± 0.8 cm H_2O after vagotomy. There was no change in IR-ANP which was 73.4 ± 4.7 pg/mL before vagotomy and 70.7 ± 4.7 pg/mL after vagotomy.

Effects of combined vagotomy and administration of atenolol

In six dogs, after vagotomy, atenolol (2 mg/kg) was given 30 and 5 min (1 mg/kg) before the test of atrial distension. The effectiveness of the cardiac β-receptor blockade was tested by injecting isoproterenol (0.5 μg/kg) before and after each dose of atenolol. Injection of isoproterenol caused an increase in heart rate of 104 ± 2.3 beats/min before giving atenolol and 7.4 ± 2.0 beats/min after giving atenolol. The changes in heart rate in response to the test dose of isoproterenol were the same after the first and second doses of atenolol. Administration of atenolol caused a decrease in mean arterial pressure from 131 ± 7.5 to 117 ± 9.7 mmHg ($p < 0.01$) and a decrease in heart rate from 178 ± 5.4 to 154 ± 7.7 beats/min ($p < 0.01$). There was a small increase in left atrial pressure from 12.6 ± 1.3 to 14.6 ± 2.0 cm H_2O; the change in left atrial pressure in one dog was much larger than in the other dogs (9 cm H_2O) so that the changes did not fall within a normal distribution. The Wilcoxon's test for pair differences gave a significance of $0.05 < p < 0.1$. There was an increase in IR-ANP from 64.2 ± 5.2 to 72.7 ± 7.7 pg/mL ($p < 0.05$). In the dog which showed a large increase in left atrial pressure IR-ANP increased by 30 pg/mL.

After vagotomy and atenolol, atrial distension did not cause significant changes in mean arterial pressure or heart rate but there was a significant increase in IR-ANP from 69.7 ± 7.6 pg/mL during the control periods to 114.1 ± 8.3 pg/mL during atrial distension ($p < 0.001$). The changes in IR-ANP induced by atrial distension after vagotomy and administration of atenolol were not significantly different from those caused by atrial distension before vagotomy. Neither vagotomy nor atenolol altered the time course of the appearance or disappearance of IR-ANP from the plasma during or after atrial distension.

Effects of stimulation of the right ansa subclavia

In six dogs, 20 min after the cervical vagus nerves had been cut, the right ansa subclavia was stimulated for 20 min. The results to these six tests are shown in Fig. 4 and compared with the effects of mitral obstruction in these dogs. Stimulation of the ansa subclavia caused a marked increase in heart rate, from 179 ± 3.7 to 272 ± 6.6 beats/min ($p < 0.01$), no significant change in mean arterial pressure and no change in mean left atrial pressure. The plasma concentration of IR-ANP was 63.2 ± 4.4 pg/mL during the control periods and 67.8 ± 5.5 pg/mL during stimulation of the ansa subclavia; this difference was not

significant. In three dogs in which blood was sampled from the coronary sinus during stimulation of the right ansa subclavia, there were no significant changes in IR-ANP in the coronary sinus plasma.

Discussion

Extracts of dog atria possess natruiretic properties similar to those of other mammalian species. The 28 amino acid peptide at the C-terminus of the precursor molecule of human ANP, which has potent natriuretic activity appears to be identical with the sequence located at the C-terminus of the dog precursor (Oikawa et al. 1985). Since the antiserum used in our experiments shows 100% cross reactivity with α-human ANP it is likely that there is a similar cross reactivity with dog ANP. We were able to show that antibodies raised to rat ANP reacted with immunoreactive substances in dog plasma and in acid extracts of dog atria (Wilson et al. 1986). The values of IR-ANP found in dog plasma, which were in the range of 45–105 pg/mL, are lower than those that have been reported in the rat. Tanaka et al. (1984) reported 156 fmol/mL (approximately 450 pg/mL) and Gutkowska et al. (1984) found 1.2–1.6 ng/mL. Both groups used an extraction for ANP immediately after sampling, lyophilization for storage, and later RIA. We did not find, as reported by Gutkowska et al. (1984) evidence of interference by plasma with the direct RIA procedure but did find that storage of plasma samples from some dogs led to progressive loss of immunoreactivity. For this reason, all values reported here were measured on plasma samples frozen immediately after centrifugation and separation, and subjected to RIA the following day. We were unable to detect any significant change in IR-ANP because of the administration of morphine, the anaesthetic agent (chloralose), or the surgical procedures. We cannot say whether the differences between the values found in dogs and those found in rats are due to the differences in the procedures used or represent a true species difference.

Mitral obstruction which raised left atrial pressure by about 10 cm H_2O caused a rapid and significant increase in IR-ANP in all dogs. This change in left atrial pressure is within the physiological range; conscious dogs have increases in left atrial pressure of 4–6 cm H_2O following a high salt meal (Kaczmarczyk et al. 1979). We have previously reported that a change in atrial pressure of this magnitude causes a decrease in plasma concentration of AVP, which is dependent upon stimulation of left atrial receptors by the increased left atrial pressure (Ledsome et al. 1983). IR-ANP could have been released from the heart by a direct effect of atrial stretch or by a neural or humoral mechanism dependent upon stimulation of left atrial receptors. We have previously presented evidence that left atrial receptors are not involved in the release of IR-ANP by atrial distension (Ledsome et al. 1985).

The time course of the changes in IR-ANP was examined in some detail because this knowledge is important in designing future experiments and in correlating expected changes in IR-ANP with physiological effects such as natriuresis. The fact that the concentration of IR-ANP in the samples from the coronary sinus was usually higher than in simultaneous samples taken from the femoral arteries and that there was a much greater increase in IR-ANP in the coronary sinus plasma during atrial distension, provides evidence that the heart was indeed the source of the IR-ANP. When there was an increase in left atrial pressure there was a very rapid release of IR-ANP from the heart; IR-ANP in the coronary sinus plasma had increased by about 50 pg/mL (Fig. 3) after 2 min and was almost doubled after 5 min, compared with a 30% increase in femoral arterial plasma. After removal of the atrial distension, the decline in IR-ANP was much slower. The disappearance of ANP followed a logarithmic decay and was highly reproducible in that the time course was the same in 12 tests done before vagotomy and in 11 tests done after vagotomy or after vagotomy plus atenolol. The decay time probably has a true value shorter than the 4.5 min calculated from our data because there was not an immediate decrease to control values of IR-ANP from the coronary sinus when atrial distension was removed. Sampling from the coronary sinus blood may provide a more sensitive index of changes in release of IR-ANP than measurements in femoral arterial blood. Although there was a much larger increase in IR-ANP in the coronary sinus plasma than in the plasma from the femoral artery, calculations based on the assumption that the coronary flow was 4% of the cardiac output and that the half life of ANP is 4.5 min, indicate that the change in the concentration of IR-ANP in the coronary sinus plasma should have been even greater to account for the change in femoral arterial IR-ANP. The reasons for this discrepancy are unknown but the discrepancy does not affect the major conclusions of this study.

Because mitral obstruction usually causes at least a transient decrease in mean arterial pressure, it was possible that during mitral obstruction there was an increase in activity in cardiac sympathetic nerves secondary to a decrease in the stimulus to arterial baroreceptors. Vagal section caused significant increases in mean arterial pressure and heart rate and did not change plasma IR-ANP concentration suggesting that neither removal of vagal tone nor reflex sympathetic activation changed plasma IR-ANP in these experiments. That the increase in IR-ANP, during atrial distension, was not due to activation of cardiac β-adrenergic receptors was confirmed by the fact that β-receptor blockade with atenolol had no effect on the increase in IR-ANP caused by atrial distension. Stimulation of the right ansa subclavia has been shown previously to cause large changes in heart rate with relatively little change in mean arterial pressure (Ledsome and Linden 1964). The heart rates reached in the present experiments approached the maximum attainable in the dog, demonstrating maximum activation of the cardiac sympathetic nerves in the right ansa subclavia. Despite the large changes in heart rate during sympathetic stimulation there were no significant changes in left atrial pressure and no changes in plasma IR-ANP. Since stimulation of the sympathetic nerves would be likely to cause activation not only of β-adrenergic receptors but also of α-adrenergic receptors present in the atria, it seems unlikely that IR-ANP can be released from the heart, under physiological conditions, by activation of either α- or β-adrenergic receptors. This is contrary to the report by Sonnenberg and Veress (1984) that activation of α receptors caused release of ANP from rat atria incubated *in vitro*. These authors also found that ANP could be released from the atria by treatment with vasopressin. This is unlikely to be the mechanism by which atrial distension releases IR-ANP since atrial distension decreases plasma AVP and since the changes in AVP associated with atrial distension are abolished by vagotomy (Ledsome et al. 1983). The finding that a large increase in heart rate induced by sympathetic stimulation did not increase plasma IR-ANP was surprising because tachycardia in both humans (Tikkanen et al. 1985) and in rabbits (Rankin et al. 1986) is associated with increases in IR-ANP. However, sympathic stimulation did not change atrial pressure, whereas atrial tachycardia and electrical pacing are both associated with increases in atrial pressure.

These results confirm that a modest increase in left atrial

pressure can cause a rapid increase in the plasma concentration of IR-ANP in blood from both the coronary sinus and the femoral artery. Removal of the stimulus of atrial stretch caused a decrease in plasma IR-ANP, which followed a logarithmic decay with a half-time of 4.5 min, and which was unaffected by vagotomy or β-adrenergic blockade. The mechanism causing release of IR-ANP is likely to be stretch of the atrial wall rather than activation of a reflex arising from stimulation of atrial receptors and activation of an efferent neural or humoral stimulus to the heart. We were unable to demonstrate release of IR-ANP secondary to stimulation of the cardiac sympathetic nerves.

Acknowledgements

This work was supported by grants from the Medical Research Council of Canada, the B. C. Heart Foundation, and the B. C. Health Care Research Foundation. The authors are grateful for the assistance of M. Greene and D. Feuchuk.

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